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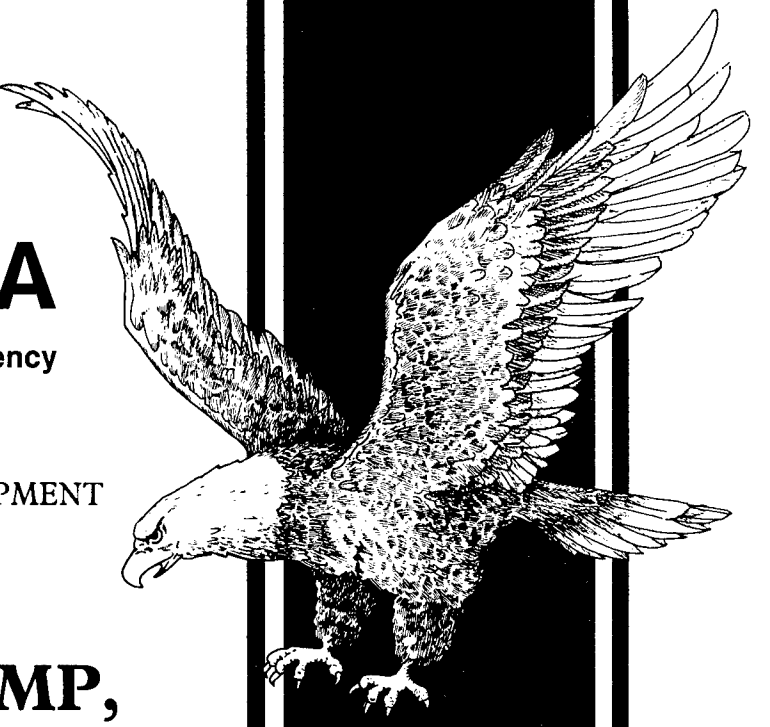

USATHAMA

U.S. Army Toxic and Hazardous Materials Agency

INSTALLATION RESTORATION PROGRAM
ENVIRONMENTAL TECHNOLOGY DEVELOPMENT

Task Order - 11 Biodegradation of DIMP, Dieldrin, Isodrin, DBCP, and PCPMSO in Rocky Mountain Arsenal Soils

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BIODEGRADATION OF DIMP, DIELDRIN, ISODRIN, DBCP, AND PCPMSO IN ROCKY MOUNTAIN ARSENAL SOILS

FINAL REPORT

Distribution Unlimited

Prepared for:

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The role of biodegradation in the environmental fate of DIMP, dieldrin, isodrin, DBCP, and PCPMSO in Rocky Mountain Arsenal soils was evaluated. Surface soil borings were collected from contaminated and uncontaminated regions at RMA. The extent of heterotrophic microbial activity in the soil samples was determined by monitoring $^{14}CO_2$ production from soils spiked with a mixture of ^{14}C-glucose, ^{14}C-acetate, and ^{14}C-amino acids. Soil extracts were plated on nutrient agar to enumerate total heterotrophs. Two of six soil samples exhibited little or no heterotrophic activity. The lack of metabolic activity in these two samples was attributed to microbially inhibitive soil contaminants. Inactive soils were not utilized in subsequent mineralization studies.

The mineralization of radiolabeled DIMP, dieldrin, isodrin, DBCP, and PCPMSO in contaminated and uncontaminated RMA soils was determined. Soil samples were spiked with test compound and incubated at 22°C. Headspace in the test flasks was periodically sampled and analyzed for ^{14}C-organics and $^{14}CO_2$. Mineralization studies were conducted for a period of 26 weeks.

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All five test compounds were poorly degraded under aerobic conditions. Low, but statisti cally significant (compared to sterile controls) levels of $^{14}CO_2$ were produced from ^{14}C-isodrin and ^{14}C-dieldrin. Previous research by other investigators indicated that th five test compounds are resistant to aerobic degradation. Data from the present study support these findings.

The mineralization of ^{14}C-isodrin was also evaluated in a laboratory-scale composting system maintained at 55°C. Less than 0.1 percent of ^{14}C-isodrin was evolved as $^{14}CO_2$ during a 39 day test period.

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SECTION 1

EXECUTIVE SUMMARY

Soils at the Rocky Mountain Arsenal (RMA) in Colorado have been contaminated with organic chemicals of environmental concern, including 1,2-dibromo-3-chloropropane (DBCP), diisopropylmethylphosphonate (DIMP), p-chlorophenylmethylsulfoxide (PCPMSO), dieldrin, and isodrin. The United States Army Toxic and Hazardous Materials Agency (USATHAMA) is investigating the ability of indigenous soil microorganisms to biodegrade these compounds to innocuous end products under RMA environmental conditions.

The capability of microorganisms indigenous to RMA soils to degrade radiolabeled test compounds was evaluated. Complete degradation (mineralization) was determined by the production of $^{14}CO_2$ from ^{14}C-labeled test compounds. Biotransformation was determined by the production of ^{14}C-metabolites from ^{14}C-labeled test compounds. The primary parameters investigated in the present study included enumeration of heterotrophic soil microorganisms and the extent of test compound mineralization in RMA soils. Experiments were conducted using both contaminated and uncontaminated RMA soils to evaluate the effects of soil contamination on the metabolic capabilities of soil microorganisms. Data obtained from these laboratory studies were used to predict the likelihood of in situ microbial removal of the contaminants of interest from RMA soils.

Six samples of surface soils collected from the vicinity of soil borings at RMA were evaluated for use in mineralization studies. Two of these soil samples (nos. 3204 and 3219) exhibited little or no microbial activity and were not used to evaluate test compound mineralization. Uncontaminated RMA soil, as well as soil nos. 3215, 3224, and 3256, contained viable populations of heterotrophic microorganisms as determined by plate counts and mineralization of a mixture of ^{14}C-glucose, ^{14}C-acetate, and ^{14}C-amino acids. However, all five test compounds were poorly degraded in these soils during 6-month studies. Low but statistically significant (compared to sterilized controls) levels of $^{14}CO_2$ were produced from ^{14}C-dieldrin and ^{14}C-isodrin only. Mineralization of ^{14}C-DIMP, ^{14}C-DBCP, and ^{14}C-PCPMSO was not statistically different in active and sterilized RMA soils at the 95-percent confidence level.

Previous research by other investigators has indicated that the five test compounds are resistant to aerobic microbial degradation and are environmentally persistent. Data from the present study support these findings. Experiments conducted to evaluate

the microbial degradation of ^{14}C-isodrin under environmentally enhanced conditions were unsuccessful. Little or no $^{14}CO_2$ was produced from ^{14}C-isodrin under aerobic composting test conditions.

SECTION 2

INTRODUCTION

Mineralization is the conversion of an organic chemical to inorganic end products such as CO_2, H_2O, N_2, and SO_4. Biodegradation and biotransformation are less precise terms that may describe either mineralization or microbial transformation to another organic compound. To determine the mineralization and biotransformation of the chemicals of interest, radiolabeled (^{14}C) test compounds were utilized in this laboratory investigation. The use of radioisotopes is the single most sensitive and precise method of evaluating the biodegradation of organic chemicals in environmental media.

The 17,000-acre Rocky Mountain Arsenal (RMA) near Denver, Colorado, has been a manufacturing site for military and agricultural chemicals for more than 30 years. As a result of these activities, numerous chemical contaminants have been detected in RMA soils and groundwater. Sources of contamination include leachate from unlined chemical storage ponds and accidental spills. Concern over the fate of these chemical contaminants increased during the 1970's when traces of DIMP and other compounds were detected in groundwater off the RMA site.

The United States Department of Defense's (DOD) mission for developing, testing, and evaluating decontamination and restoration technologies has been assigned to USATHAMA. Environmental Science and Engineering (ESE, Denver, Colorado) and Ebasco (Santa Ana, California) were operating under USATHAMA contract to conduct a comprehensive remedial investigation for RMA on a grid-by-grid basis. WESTON was under USATHAMA contract to study the biodegradability of 1,2-dibromo-3-chloropropane (DBCP), diisopropylmethylphosphonate (DIMP), p-chlorophenylmethylsulfoxide (PCPMSO), isodrin, and dieldrin under RMA-specific conditions. WESTON was charged with determining whether long-term exposure of soil microorganisms to these chemicals has led to microbial adaptation to these contaminants and enhanced the biological removal of these compounds at the RMA site.

Shell Chemical Company previously manufactured DBCP, dieldrin, and isodrin within its currently inactive RMA production facility. DIMP is a by-product of military chemical production, and PCPMSO is a by-product of previous commercial chemical production. Shell and the U.S. government are currently involved in litigation regarding the disposition of these and other environmental contaminants at RMA.

ESE and Ebasco analytical data indicate that soil contaminants exist in localized pockets, with a great deal of vertical and horizontal variability. These data dictated that biodegradation

experiments on each ^{14}C compound studied by WESTON would have to be investigated in discrete, well-characterized soil samples. Contaminated soils used in biodegradation studies were documented previously as containing the specific contaminant to be tested. Since no single soil contained all five contaminants, background parameters among experiments were not considered to be equivalent.

2.1 LITERATURE REVIEW

The available literature indicates that the five compounds of interest are relatively resistant to microbial degradation (Table 2-1). The long-term persistence of the compounds in RMA soils supports this conclusion. While the environmental fate of DBCP and dieldrin has been studied fairly extensively in various environments, little experimental data exist concerning the fate of PCPMSO, DIMP, and isodrin outside of the RMA geographical area.

2.1.1 DBCP

DBCP is an industrial nematocide marketed commercially as Nemagon® and is used as a soil fumigant. Environmental persistence (resistance to microbial attack) is a critical property for pesticides, and this property has enhanced the agricultural utility of DBCP. Volatilization is perhaps the foremost mechanism affecting the fate of DBCP in soils. DBCP was found to be much more persistent when injected into soils rather than applied to soils in irrigation water (Hodges and Lear, 1974).

Nelson et al. (1981) investigated DBCP contamination of groundwater and subsurface soils in the San Joaquin Valley of California and found that:

- DBCP was detected in subsurface soils at ppb levels.
- Clays and silts absorbed more DBCP than sands, but downward vertical migration of DBCP was observed regardless of soil type.
- DBCP was detectable in topsoil at one site 6 years after a single application.

DBCP reached the groundwater below peach orchards in South Carolina after fumigation for nematode control (Carter et al., 1984).

At a concentration of 1 percent in soil, DBCP had the following effects on the activity and population level of a soil microbial community (Berkowitz et al., 1978):

- Reduced the microbial population and eliminated the release of CO_2, suggesting that DBCP inhibited the respiration of, but did not eliminate, the microflora.

Table 2-1

Properties of Dieldrin, Isodrin, DBCP, DIMP, and PCPMSO

Compound	Water Solubility (ppm)	Vapor Pressure (mm Hg)	Estimated Half-Life (years)
Dieldrin[1]	≤ 1	$\leq 10^{-4}$	≥ 5
Isodrin[1]	≤ 1	$\leq 10^{-4}$	≥ 5
DIMP[2]	$\geq 10^3$	$10^{-4} \leq VP \leq 1$	> 2[3]
DBCP[1]	$1 \leq S \leq 10^3$	$10^{-4} \leq VP \leq 1$	0.5-1
PCPMSO[1]	$10^3 \leq S \leq 5 \times 10^4$	$10^{-4} \leq VP \leq 1$	0.5-1

[1]After Cogley and Foy, 1978.
[2]After Rosenblatt et al., 1975.
[3]After Spangord et al., 1979.

- Reduced the numbers of proteolytic bacteria by 53 percent, cellulytic bacteria by 97 percent, and nitrifiers by 100 percent.

- Reduced nitrification by almost 50 percent.

Dehalogenation of DBCP has been observed in soil/water cultures (Castro and Belser, 1968). Halide release from DBCP occurred only during the microbial growth phase. The presence of an additional organic carbon source (glycerol) was also required, suggesting cometabolic transformation of DBCP. Biotin and thyamine were also added to the test soil. A maximum of 63-percent transformation was observed after 4 weeks of incubation at pH 8. The authors also screened approximately 100 soils from southern California orchards and fields for the ability to dehalogenate DBCP. Of these, approximately 75 percent showed some capability to bring about the release of bromide. However, efforts to isolate a pure culture capable of DBCP dehalogenation were not successful (Castro and Belser, 1968).

2.1.2 Dieldrin

Dieldrin is part of the group of cyclodiene insecticides that includes isodrin, aldrin, and endrin. Though no longer registered for general use, dieldrin was used extensively in the past to protect corn crops against insects and wood products against termites.

Dieldrin has been found to be extremely persistent in soils under aerobic and anaerobic conditions (Castro and Yoshida, 1971; Spangord et al., 1977) and was not biodegraded in standard screening tests or in river waters (Tabak, 1981; Eichelberger and Lichtenberg, 1981; Sharom, 1980).

Nash and Woolson (1967) conducted a field study to determine the persistence of dieldrin in Congaree sandy loam soil. The soils received dieldrin applications equivalent to 0 to 448 kilograms of insecticide per hectare at the start of the study. Treatment and maintenance of the field plots was such that loss of dieldrin by volatilization, leaching, photolysis, and mechanical removal was minimized. High dosage rates were considered toxic to the microflora. After 15 years, approximately 31 percent of the original technical-grade dieldrin remained in both high (100 ppm) and low (25 ppm) concentration plots.

Twelve out of 650 soil microorganisms, isolated from a site contaminated heavily with pesticides, degraded dieldrin (Matsumura and Boush, 1967). The isolated organisms included six species of Pseudomonas and two Trichoderma species. Ketone, aldehyde, and alcohol derivatives of dieldrin were produced by one of the most active microbes. Other studies indicated that a

major product of microbially mediated transformation of dieldrin was photodieldrin (Vockel and Korte, 1974; Matsumura et al., 1970). It has been reported that the fungus Trichoderma koningii converted dieldrin to carbon dioxide; however, only 3 percent of the dieldrin initially present was converted to CO_2 (Bixby et al., 1971).

Dieldrin and photodieldrin were found to resist microbial degradation in several other studies. Halvorsen et al. (1979) reported no degradation of dieldrin in sewage lagoon sediment. Thirty-five out of 100 marine phytoplankton cultures were capable of transforming dieldrin to photodieldrin (Matsumura and Boush, 1972). Photodieldrin has been reported to be resistant to degradation by algae (Reddy and Khan, 1975). Meuller and Korte (1972) reported on the degradation of dieldrin by composting. After 3 weeks, 97 percent of the initial dieldrin spike was recovered as parent compound, indicating that little microbial transformation had occurred.

2.1.3 DIMP

DIMP is a by-product formed during the synthesis of the nerve agent isopropylmethylphosphorofluoridate (Sarin). Few studies exist that directly address the biodegradation of DIMP. Spangord et al. (1979) evaluated the rate and extent of microbial and photochemical transformation of DIMP in RMA soil and water samples. Biotransformation of DIMP in natural waters did not occur despite attempts to acclimate the test microorganisms. In acclimated soil, ^{14}C-DIMP was converted to $^{14}CO_2$ to the extent of only 5 percent after 17 weeks of incubation at 25°C. Transformation was essentially nonexistent at 10°C. A half-life of greater than 2 years was predicted for ^{14}C-DIMP in soil.

Vertical migration of DIMP through soil has been observed in soil lysimeter studies using soils from locations other than RMA (O'Donovan and Woodward, 1977). DIMP was applied to the soil by two different methods. The first method involved chronic application of a 20-ppm solution of DIMP in distilled water to the top of soil columns. This application method resulted in a thin layer of soil at the top of the column that had a relatively high DIMP concentration (18 to 33 ppm). DIMP was detected at lower concentrations (3 to 9 ppm) throughout the remainder of the soil profile. The second application method consisted of spiking the upper 1 foot of the soil columns with 20 ppm of DIMP and flowing distilled water through the columns. This method resulted in a slightly broadened band of DIMP leaching through the soil column. Originally present at 20 ppm in the upper 1 foot of soil, DIMP was not detectable in the upper 2 feet of soil in several experiments. In the other experiments, DIMP was also not detected at greater depths. These data suggest that DIMP is relatively mobile in soil (O'Donovan and Woodward, 1977). The reported water solubility of DIMP supports this statement (Table 2-1).

Existing data suggest that DIMP is semivolatile in soil (O'Donovan and Woodward, 1977; Spangord et al., 1979). In a series of volatilization studies, O'Donovan and Woodward (1977) measured the evaporative loss of radiolabeled DIMP from soil columns spiked with 20 ppm of ^{14}C-DIMP. Volatilization of DIMP from dry soil was measured in columns that were 25 millimeters wide and 100 centimeters deep. After the columns were subjected to an air flow of 100 milliliters per minute passing across their surfaces for 250 hours, the dry and moist soils retained greater than 95 percent and approximately 78 percent of the radioactivity originally present, respectively.

The presence of a nonreactive C-P bond contributes to the environmental persistence of DIMP and related compounds (Daughton et al., 1979a; Yerweij et al., 1976). While organophosphorus pesticides are generally considered nonpersistent because of the susceptibility of phosphoesters to hydrolysis (Daughton et al., 1979b), compounds that contain C-P bonds are known to be resistant to hydrolysis, photolysis, thermal degradation, and microbial degradation (Yasu and Roy, 1985).

There is no evidence for biological breakdown of C-P bonds under environmental conditions where an abundance of phosphate is present (Yerweij et al., 1976). Only a few microorganisms have been cited as capable of utilizing methyl phosphonic acid (MPA), which contains the C-P bond. Zeleznick et al. (1963) determined that Escherichia coli Cookes strain was able to utilize MPA (6.1 g/L) and ethyl phosphonic acid (EPA) (6.8 g/L) when these compounds were present as the sole phosphorus source. The total phosphorus content of the bacterial pellets increased approximately ten-fold after 24 hours of incubation with MPA as the sole phosphorus source (Zeleznick et al., 1963).

Daughton et al. (1979a, 1979b) detected the cleavage of C-P bonds during the catabolism of O-alkyl alkylphosphonates by Pseudomonas testosteroni. Under aerobic conditions with O-alkyl alkylphosphonates as the sole source of phosphorus (concentrations of 0.008 to 0.10 millimoles), P. testosteroni cleaved the C-P bonds and transformed the organophosphorus toxicants to innocuous end products. P. testosteroni was capable of growth with ionic MPA, O-IMP, EPA, or O-EPA as the sole phosphorus source. Alkanes were produced from the C-P bond cleavage. This work was the first to establish:

- Biodegradation of a simple alkylphosphonate.
- Complete conversion of an organophosphorus toxicant to innocuous end products.
- The catabolic pathway for alkylphosphonate.

2.1.4 Isodrin

Isodrin is a member of the cyclodiene group of insecticides that includes aldrin, dieldrin, and endrin and was formerly marketed under the trade name Telodrin®. Isodrin is an intermediate in the synthesis of endrin (isodrin is the epoxide of endrin) and is an isomer of aldrin. Isodrin is made by the slow reaction of cyclopentadiene with the condensation product of vinyl chloride and hexacholorocyclopentadiene (Brooks, 1974).

Because isodrin was not widely used as an insecticide, data on the compound's biodegradability and environmental fate are limited and are usually inferred indirectly from studies of other cyclodienes. No data from laboratory or field studies concerning the microbial degradation of isodrin were found in the open literature. However, biodegradation of other cyclodiene insecticides has been well documented. Because of the close structural relationship of isodrin to aldrin and dieldrin, a brief summary of the biodegradation of these cyclodiene compounds is presented here.

Biotransformation of aldrin has been reported in numerous studies. Aldrin was degraded to unidentified end products by Chlorella purenoidose (Elsner et al., 1977). Dieldrin and ketoaldrin were produced from the degradation of aldrin by Penicilium funiculosum (Murado-Garcia, 1973). Ninety-two microbial cultures were isolated from soil that degraded aldrin to dieldrin and other unidentified metabolities (Tu et al., 1968). Thirteen out of 20 dieldrin-degrading organisms also transformed aldrin, with the major transformation product being 6,7-dihydroxyaldrin (Patil and Matsumura, 1970). The epoxification of aldrin to dieldrin in soil is reportedly carried out more effectively by fungi and actinomycetes than bacteria (Tu et al., 1968).

Of a series of 150 soil cultures, 25 were able to convert endrin to ketoendrin (Matsumura et al., 1971). Twenty dieldrin-degrading cultures also transformed endrin to ketoendrin (Patil and Matsumura, 1970).

Extrapolation of complete cyclodiene transformation in the field from laboratory data is tenuous (Crowley and Lichtenstein, 1970). Partial degradation of cyclodienes has been observed under some field conditions; aldrin disappeared more rapidly in upland soil conditions than in flooded conditions (Castro and Yoshida, 1971). The increased persistence of aldrin in flooded soils was attributed to a lack of molecular oxygen, which is required for the epoxidation of aldrin. Endrin was also persistent in flooded soils, with the exception of Casiguian soil (Castro and Yoshida, 1971).

When 100 samples of estuarine and oceanic surface films, marine plankton, and algae were incubated with cyclodienes, 35 out of 100 estuarine cultures were able to degrade aldrin, dieldrin, and endrin to trans-aldrindiol, photodieldrin, and ketoendrin (Matsumura and Boush, 1972). Open-ocean water samples proved to be less active. Ocean sediments were ineffective at transforming cyclodienes, indicating that the presence of algae was necessary to effect transformation.

Extreme functional persistence of cyclodiene insecticides, including isodrin, has been observed in soils. The persistence of these compounds in soil has been attributed to the highly stable ring structure and the toxicity of the few known transformation products. Isodrin has an estimated half-life in soil of 4 to 8 years, forming degradation products that include endrin (TOXNET, 1987). Nash and Harris (1973) reported that 16 percent of the isodrin and 39 percent of the endrin applied to soils during a long-term persistence study remained after 16 years.

Despite the low water solubility and relative immobility of cyclodiene insecticides, considerable transport of cyclodienes eventually occurs under field conditions because of the great persistence these compounds exhibit. The relative mobilities of chlorinated hydrocarbons, including cyclodienes, as reported by Nash and Woolson (1968) in sandy loam soil were: lindane > isodrin > heptachlor > endrin > toxaphene > dieldrin > aldrin > dilan > chlordane.

2.1.5 PCPMSO

P-chlorophenylmethylsulfoxide (PSPMSO) is produced in trace amounts as an intermediate in the production of the herbicide Planavin®. PCPMSO is formed from the incomplete tungstate-catalyzed oxidation of the corresponding p-chlorophenylmethylsulfide (PCPMS) with hydrogren peroxide (Sanderson and Swift, 1972). The authors postulated that another possible source of PCPMSO may be the oxidation by hydroperoxides and biochemical pathways in soil (Bateman and Hardgrove, 1954 a, b; Overberger and Cummins, 1953 a, b; Kresze et al., 1965). Guensi et al. (1979) evaluated the degradation of PCPMSO in a 160-day laboratory study. Total CO_2 evolution from the soil was used as an indicator of the broad effects of the PCPMSO on soil microbial populations. Concentrations of 0.5 and 5.0 ppm PCPMSO had no significant effect on total CO_2 evolution. A PCPMSO concentration of 50.0 ppm lowered CO_2 evolution by 8.2 percent. The evolution and recovery of $^{14}CO_2$ was used to quantify the ring cleavage and subsequent oxidation of ^{14}C-PCPMSO. After 160 days of incubation, 16.1, 10.7, and 6.1 percent of the initial ^{14}C-PCPMSO was recovered as $^{14}CO_2$ from test flasks that contained 0.5, 5.0, and 50.0 ppm of PCPMSO, respectively.

2.2 OBJECTIVES

The primary objective of this laboratory investigation was to determine the ability of microorganisms indigenous to RMA soil to biodegrade DBCP, DIMP, PCPMSO, isodrin, and dieldrin under RMA-specific conditions. Secondary objectives included the following:

- Determine whether indigenous RMA microorganisms have undergone adaptation to the soil contaminants such that enhanced in situ biotransformation of the test compounds is occurring in contaminated RMA soils.
- Determine the rate and extent of biotransformation of the five test compounds in soil under RMA-specific conditions.
- Identify and quantify the organic biotransformation products produced as a result of microbial degradation of the test compounds and determine whether these compounds have been identified previously as contaminants of unknown origin in RMA soils.
- Evaluate the effect of soil contamination on microbial activity in RMA soils.

SECTION 3

METHODS AND MATERIALS

3.1 SOIL SAMPLE COLLECTION AND STORAGE

Surface soil samples were collected from RMA by ESE and Ebasco personnel. Grab samples were collected in the vicinity of test boreholes whose chemical contaminants had been characterized previously. Soil samples were collected using USATHAMA soil boring methods and were immediately shipped to WESTON's Fate and Effects Laboratory on ice in insulated coolers using chain-of-custody procedures. Upon receipt, samples were logged in and pertinent information was recorded in a dedicated laboratory notebook. Samples collected from the vicinity of borehole nos. 3224, 3219, and 3256, as well as from uncontaminated regions, were received in polybutyrate coring tubes, homogenized in clean stainless steel buckets, and stored in glass jars in a solvent-free refrigerator. Samples from borehole nos. 3204 and 3215 were received and stored in the solvent-free refrigerator in sealable 5-gallon plastic buckets.

3.2 SOIL CHARACTERIZATION

All RMA soil samples were analyzed for pH, soil moisture content, water holding capacity (WHC), total organic carbon (TOC), total kjeldahl nitrogen (TKN), and total phosphate (TP).

3.2.1 Soil pH

Soil pH was determined by placing 20 grams of soil in a 100-ml beaker with 20 milliliters of deionized water, stirring the suspension every 10 minutes for 30 minutes, allowing the suspension to settle for 1 hour, and measuring the pH of the liquid by electrode.

3.2.2 Soil Moisture

Soil moisture was determined by weight loss. Approximately 20 grams of soil was placed in a tared beaker, and the soil mass was determined and recorded immediately. The sample was then placed in a 104°C oven until a constant weight was recorded. Soil moisture was calculated from the dry and wet weights of the samples using Equation 3.2.

$$\text{Moisture content (wt \%)} = [(\text{mass moisture})/(\text{mass dry soil})] \times 100$$

Equation 3.2

3.2.3 Water Holding Capacity

Water holding capacity (WHC) was measured by determining the mass of water retained by a dried soil sample of known mass contained in a funnel lined with filter paper and was calculated using Equation 3.3.

$$WHC(g/g) = \frac{\left[\left(\begin{array}{c}\text{mass wet}\\ \text{filter paper}\end{array} + \begin{array}{c}\text{mass}\\ \text{wet soil}\end{array}\right) - \left(\begin{array}{c}\text{mass wet}\\ \text{filter paper}\end{array}\right) - \left(\begin{array}{c}\text{mass}\\ \text{dry soil}\end{array}\right)\right]}{(\text{mass dry soil})}$$

Equation 3.3

3.2.4 Soil Characterization

Methods used to determine the TOC, TKN, and TP of the soils were derived from U.S. EPA Test Methods for Evaluating Solid Waste (U.S. EPA SW46).

3.2.5 Contaminant Characterization

RMA soil sample nos. 3204, 3215, 3219, 3224, and 3256 and uncontaminated RMA soil were analyzed for DIMP, isodrin, dieldrin, DBCP, and PCPMSO. Methods used to quantify organic contaminants and radiolabeled transformation products in RMA soils are presented in Appendix A (Analytical Methods).

3.3 ENUMERATION OF MICROORGANISMS

3.3.1 Extraction of Microorganisms from Soil Samples

Microorganisms were extracted from soil samples to provide inoculum for ^{14}C-MPN enumerations and direct plate count determinations. A 10-g soil aliquot was placed in a autoclaved dilution bottle with 90 milliliters of filter-sterilized 0.1M $NH_4H_2PO_4$ and five drops of Tween 80 (polyoxyethelene sorbitan monoleate). The mixture was agitated for 20 minutes on a wrist action shaker to promote the release of soil-bound microorganisms. Coarse sand and soil particles were allowed to settle out before the extract was removed and enumerated.

3.3.2 ^{14}C Most-Probable Number Enumeration

The population densities of heterotrophic bacteria in the soil samples from borehole nos. 3219, 3224, and 3256 were estimated by the MPN enumeration technique of Lehmicke et al. (1979) using a mixture of ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate as substrate.

Serial dilutions of soil extract were incubated with the radiolabeled substrate in biovials placed in scintillation vials that contained CO_2 trapping solution. The trapping solution was analyzed for $^{14}CO_2$ in a Tracor (model 6905) liquid scintillation counter after 60 days of incubation using the

counting procedure described in Subsection 3.4.1. The most-probable number of heterotrophic bacteria in each soil extract was estimated using standard MPN tables.

3.3.3 Plate Count Enumeration

Estimates of the total heterotrophic bacterial populations in all RMA soils were made by plating serial dilutions of soil extract on nutrient agar. Plates that contained between 30 and 200 bacterial colonies were used to calculate the number of colony-forming units (CFU) per gram of soil.

3.3.4 Soil Metabolic Potential

The metabolic status of heterotrophic bacterial populations in RMA soils was determined by monitoring the mineralization of a mixture of ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate in soil over a period of 21 days. The experiments were conducted in nonvolatile compound test flasks using the methods described in Subsection 3.4.1.

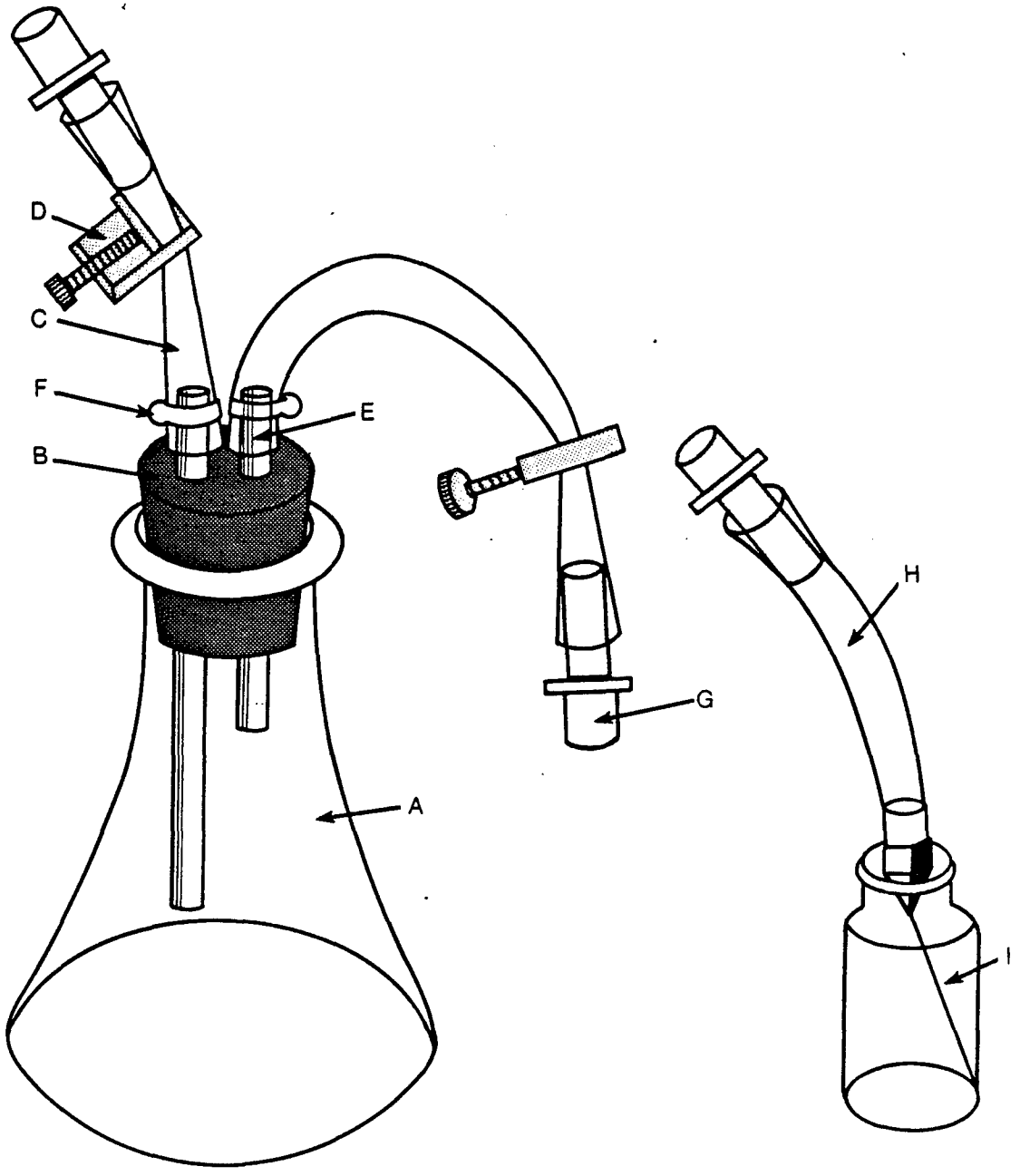
3.4 MINERALIZATION EXPERIMENTS

The general procedures for conducting mineralization rate studies are presented in the following subsection. In some cases, the chemical and physical properties of the five test compounds required slight modification of the test procedures. The modifications employed for specific compounds are explained at the end of this subsection.

3.4.1 Nonvolatile Compounds

The mineralization rate of nonvolatile isodrin and the metabolic potential of all RMA soils were determined using 125-ml Erlenmeyer flasks sealed with a no. 6 two-hole rubber stopper. Six-inch lengths of Tygon tubing (3/16-in. interior diameter, 5/16-in. exterior diameter) attached to two pieces of glass tubing (5-mm interior diameter) were fitted into the stopper holes and served as inflow and exhaust ports. The Tygon tubing was fitted with quick-disconnect plugs for attachment to a gassing manifold and exhaust tube. The exhaust tube was connected to a hypodermic needle that was placed in a scintillation vial filled with trapping solution during sampling (Figure 3-1).

Flasks were sterilized prior to use by autoclaving them for 20 minutes at 121°C and 15 psi. Approximately 800,000 dpm of ^{14}C-test compound, in a stock solution of water or acetone, was pipetted into the bottom of the flasks. Filter-sterilized deionized water was added to the flasks, if necessary, to bring the soil up to 60-percent water holding capacity. One-percent sodium azide solution was added to sterile flasks instead of



A - 125 ml Erlenmeyer flask
B - #6 Two-hole stopper
C - 2/16 I.D., 5/16 O.D. tygon tubing
D - Fixed jaw tubing clamp
E - 5 mm O.D. glass tubing
F - Ty-rap cable tie
G - Quick disconnect plug
H - Exhaust tube with syringe needle
I - Scintillation vial

FIGURE 3-1 NONVOLATILE COMPOUND TEST FLASK

sterile deionized water. Thirty grams of test soil was aseptically transferred to each test flask, and the flasks were sealed with the rubber stoppers.

Experiments were conducted using two sets of triplicate microcosms of active contaminated and uncontaminated soils and a single sterile control microcosm for each soil type (eight flasks per set). One set of flasks was sacrificed at time zero, and the other set was sacrificed after 26 weeks of incubation and headspace sampling.

Sampling was accomplished by hooking the inflow tubing to a specially designed gassing manifold, placing the hypodermic needle on the end of the exhaust tube into a scintillation vial that contained 10 milliliters of trapping fluid, and purging the gas inside the flask through the trapping fluid for 30 minutes. The manifold was designed to produce a flow of 33 ml/minute through the flasks. Compressed air was passed through a series of scrubber bottles that contained 10 N NaOH to remove CO_2 before the airstream entered the test flasks (Figure 3-2). The trapping fluid consisted of a mixture of seven parts methoxyethanol to one part monoethanol amine (v/v). After sampling was completed, each scintillation vial received 10 milliliters of PCS scintillation cocktail and was refrigerated in the dark for at least 24 hours to reduce chemiluminescence.

Radioactivity in the sample vials was quantified by counting the scintillation vials for 2 minutes in a Tracor (model 6905) liquid scintillation counter. Three background vials that contained only trapping solution and scintillation cocktail (no ^{14}C) were included in each batch of samples. The scintillation counter was programmed to count the three background vials first, average the activity, and subtract this value from subsequent samples. Counts were automatically converted from cpm to dpm by the counter. The isodrin mineralization study was conducted using these procedures.

3.4.1.1 Mass Balance of Isodrin

Eight isodrin test flasks were sacrificed at the start of the experiment. Three flasks each, containing uncontaminated and contaminated soil with one sterile control for each soil type, were submitted for extraction and GC/MS-GFPC immediately after spiking with ^{14}C-isodrin. These time-zero flasks were not purged on the nonvolatile sampling apparatus. After solvent extraction, 0.5-gram samples from each test flask were combusted in a Lindberg combustion furnace, and the resulting $^{14}CO_2$ produced was trapped in 50 milliliters of CO_2 trapping solution (see Subsection 3.4.1). Ten milliliters of the trapping solution were added to 10 milliliters of PCS scintillation cocktail (National Diagnostics) in a liquid scintillation vial, and the radioactivity of each scintillation vial was

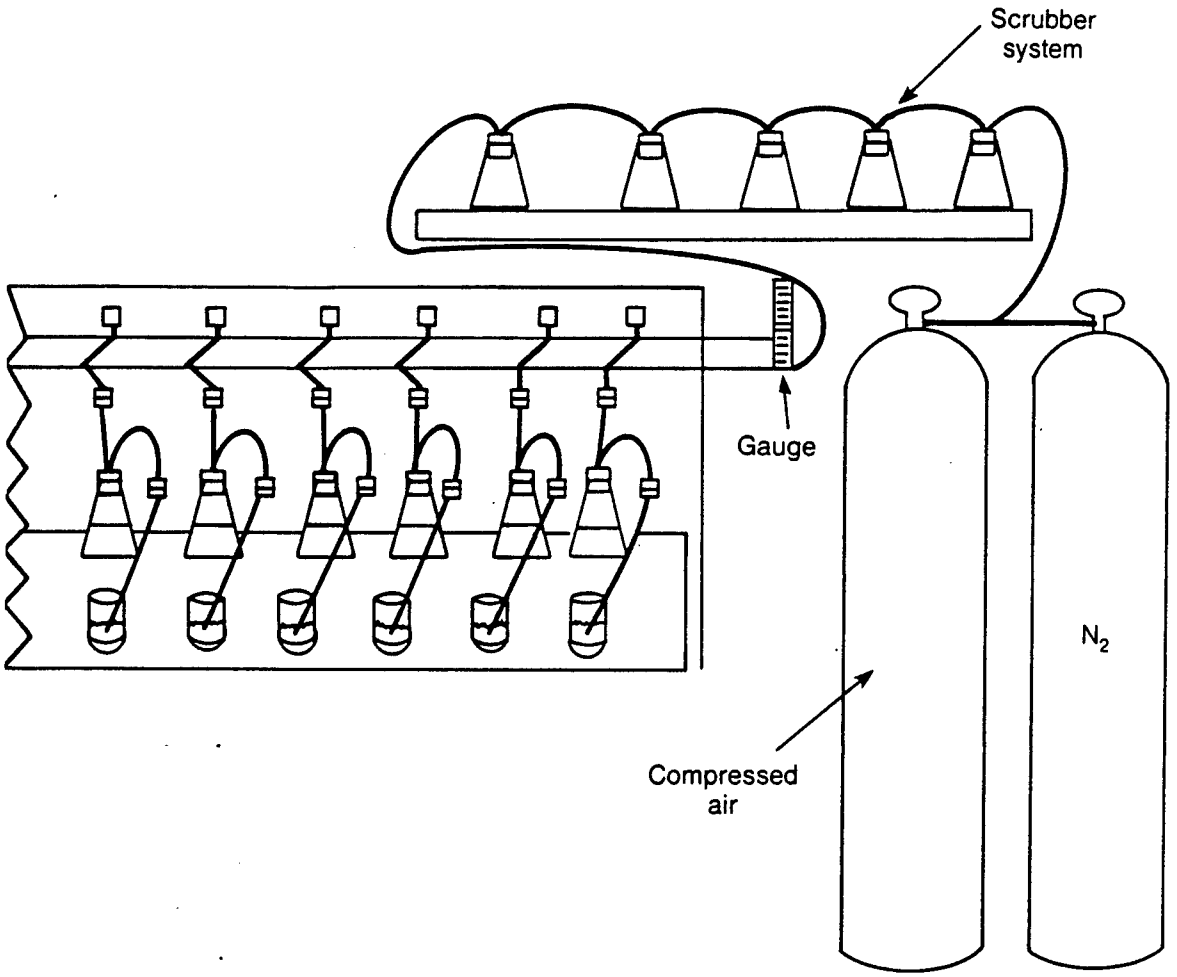


FIGURE 3-2 NONVOLATILE COMPOUND GASSING MANIFOLD SYSTEM

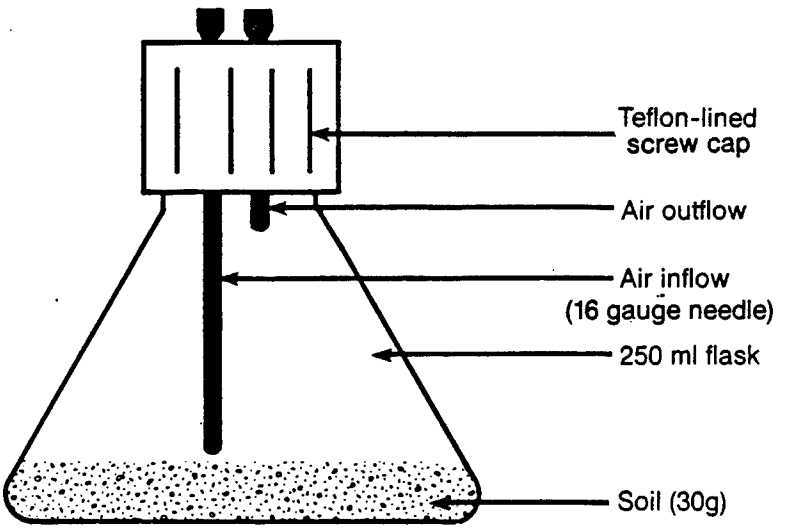
quantified by LSC. The mass balance of ^{14}C-isodrin in time-zero flasks was calculated from the amounts of radioactivity detected as solvent-extractable and nonsolvent-extractable ^{14}C in each test flask.

The mass balances for ^{14}C-isodrin in the eight test flasks that were sampled for $^{14}CO_2$ over time were determined as follows. Radioactivity extracted during periodic headspace sampling was recorded, and spreadsheets indicating cumulative percent $^{14}CO_2$ evolved were maintained. At the termination of a given experiment, test flasks were acidified with 10 milliliters of 1 N HCl, attached to the nonvolatile sampling apparatus, and purged for 30 minutes to sample the entire volume of headspace. The radioactivity levels obtained in these samplings were added to the spreadsheets, and the total amount of $^{14}CO_2$ evolved over the duration of the experiment was calculated. Plots of percent mineralization versus time were generated by calculating the mean percent test compound mineralization and 95-percent confidence intervals in triplicate microcosms and plotting these values against time.

Test soils were subsequently subjected to solvent extraction, and the concentrated extracts were analyzed for ^{14}C-parent compound and ^{14}C-organic biotransformation products by gas chromatography/mass spectrometry gas-flow proportional counting (GC/MSGFPC). The analytical methods used are presented in Appendix A. Triplicate samples of the post-extraction soil from each test flask were combusted in a Lindberg combustion furnace. The radioactivity recovered as $^{14}CO_2$ from the combustion was determined by liquid scintillation counting, and the total amount of radioactivity remaining in each test flask after extraction was calculated. This value was incorporated into the mass balance for each test flask. The mass balance of ^{14}C-test compound in each test flask was calculated by summing the radioactivity detected during periodic samplings, the final GFPC analysis, and the post-extraction combustion. Total percent test compound recovery was calculated by dividing the total recovered activity by the activity initially added to each flask.

3.4.2 Volatile Compound Mineralization Studies

The extent and rate of volatile compound mineralization were determined using 250-ml screw-cap Erlenmeyer flasks as test microcosms. Screw caps were lined with Teflon septa. Two hypodermic needles were inserted through the caps and cemented in place with epoxy cement to serve as sampling ports (Figure 3-3). Six sets of eight test flasks each were utilized for the DIMP, DBCP, and PCPMSO experiments. Each set of test flasks consisted of triplicate flasks that contained uncontaminated and contaminated soil, with one sterile control per soil type. One



Source: Marinucci and Bartha, 1979

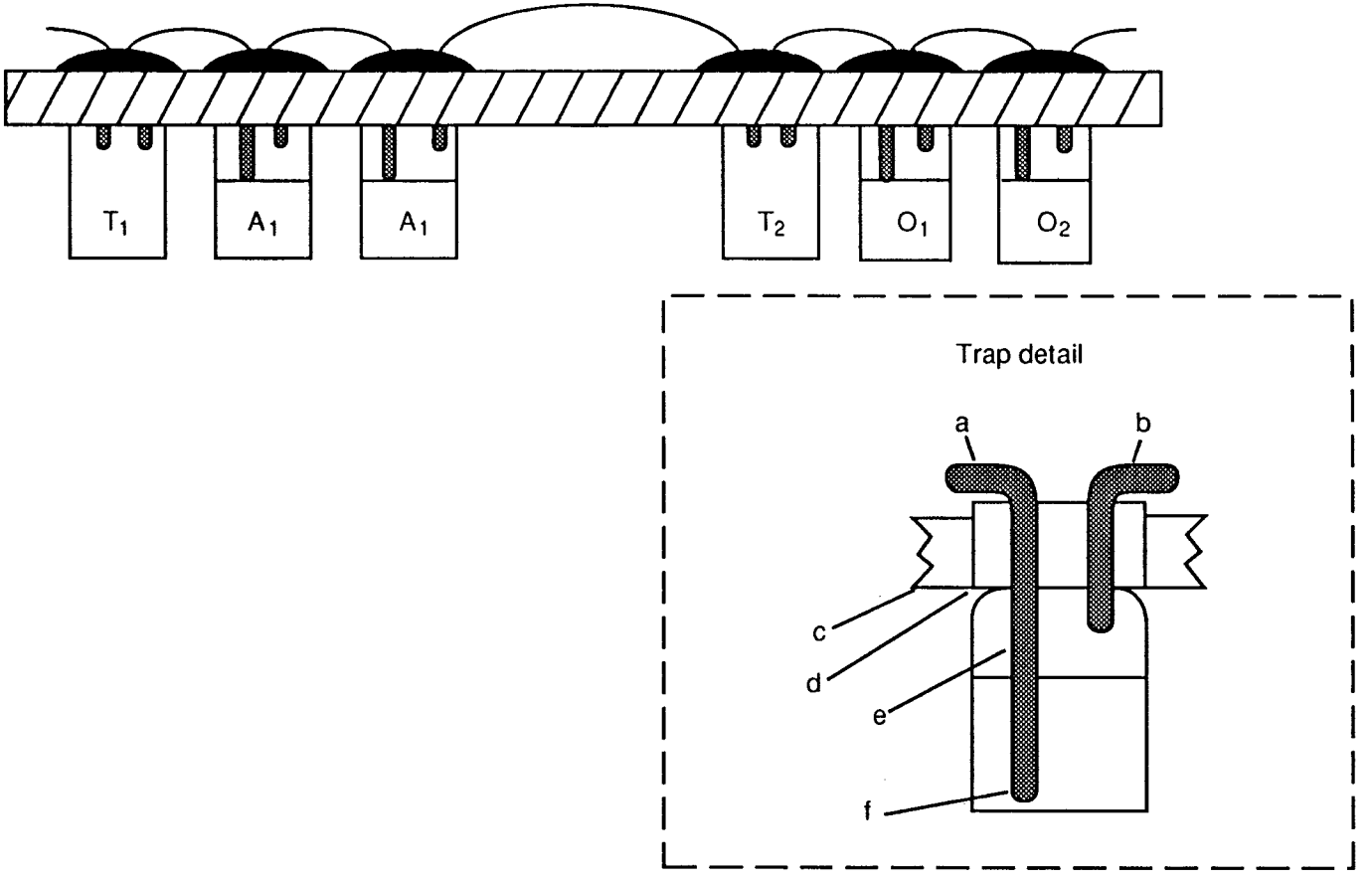
FIGURE 3-3 VOLATILE COMPOUND TEST FLASK

set of the test flasks was sacrificed at weeks 0, 2, 5, 10, 18, and 26 of the study. All test flasks were sampled weekly until the specified time of their sacrifice.

Flasks were sterilized prior to use by autoclaving them for 20 minutes at 121°C and 15 psi. Thirty grams of test soil was aseptically transferred to each test flask, and filter-sterilized deionized water was added to the soil, if necessary, to bring the soil to 60-percent water holding capacity. Soil placed in flasks designated to be sterile controls was autoclaved at 121°C for 20 minutes. One-percent sodium azide solution was added to sterile flasks instead of sterile deionized water. After sealing the flasks, approximately 800,000 dpm of ^{14}C-test compound, in a stock solution of acetone, was injected into the flasks through one of the sampling ports by a 100-ul syringe. Stock solutions of ^{14}C-test compounds were assayed to determine the exact amount of activity injected into test flasks. Flasks were agitated to distribute the test compound more uniformly throughout the soil. One sampling port on each flask was sealed with an epoxy-filled syringe body, and the other sampling port was sealed with a stainless steel syringe stopcock (Becton-Dickinson, model 3152) to facilitate headspace sampling. Experiments were conducted using triplicate microcosms of active contaminated and uncontaminated soils and a single sterile control microcosm for each soil type.

Headspace contained within the test flasks was sampled periodically to provide oxygen to the microcosms and to quantify mineralization and volatilization of test compound. A 10-ml gas-tight syringe (Hamilton) with a stainless steel stopcock attached to the end was sealed to the stopcock on the flask. The two stopcocks were opened, and 3 milliliters of headspace was withdrawn from each microcosm by gas-tight syringe and injected into the sampling port of a specially designed volatile compound sampling apparatus (Figure 3-4). The gas sample was drawn through a series of six scintillation vial traps by negative pressure. The second and third vials (from the sampling port) in the series contained 15 milliliters of Betafluor liquid scintillation cocktail (National Diagnostics) to trap undegraded parent compound and organic biotransformation products. The fifth and sixth trapping vials contained 15 milliliters of Oxasol liquid scintillation cocktail (National Diagnostics) to trap $^{14}CO_2$. The first and fourth traps contained no trapping solution and served only to catch backflow from the trapping vials.

The traps were bubbled for approximately 10 minutes to allow the entire volume of gas sample to be drawn through the sampling train. The trapping vials were removed from the sampling train, capped, refrigerated in the dark for at least 24 hours, and counted in the liquid scintillation counter as described in



Source: Marinucci and Bartha, 1979.

T_1 and T_2 are backflow traps. A_1 and A_2 contain betafluor for volatile organic trapping. O_1 and O_2 contain Oxasol for CO_2 trapping. Trap detail: (a and b) inflow and outflow (1/16 in. O.D.); (c) wood mounting board; (d) scintillation vial caps; (e) glass scintillation vial; (f) trapping fluor.

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Figure 3-4. Volatile compound sampling apparatus.

Subsection 3.4.1. Headspace removed from each vial during sampling was replaced with an equal volume of pure oxygen to maintain oxygen tension and ambient pressure in the microcosms. The DIMP, DBCP, and PCPMSO mineralization studies were conducted using these procedures.

Because of the possibility of some volatilization of dieldrin during the mineralization study, the study was conducted in volatile compound flasks using a modified sampling procedure. Two sets of eight test flasks each were utilized in the study, one set for sacrifice at time zero and one set for sacrifice after 26 weeks of incubation and sampling. Instead of sampling a small volume of headspace by gas-tight syringes as was done in the DIMP, DBCP, and PCPMSO studies, each dieldrin test flask was attached to the volatile compound sampling apparatus and purged for 30 minutes. Any volatilized organics would have been retained in the organic trapping fluid. The sampling traps were counted in the same manner as those from the DIMP, DBCP, and PCPMSO studies.

3.4.2.1 Calculation of Volatile Test Compound Mass Balance

Eight test flasks were sacrificed at the start of the DIMP, DBCP, and PCPMSO mineralization studies. These time-zero flasks consisted of three each that contained uncontaminated and contaminated soil, with one sterile control for each soil type. Immediately after spiking with ^{14}C-test compound, the time-zero flasks were hooked up to the volatile compound sampling apparatus and purged for 30 minutes (see Subsection 3.4.2). Ten milliliters of 1 N HCl were added to each flask, and they were sampled for an additional 30 minutes using new trapping solutions.

After purging, the time-zero test flasks were submitted for extraction and GC/MS-GFPC analyses. Post-extraction soil samples were combusted to quantify nonextractable ^{14}C activity.

To calculate the mass balance of ^{14}C in time-zero test flasks, the total quantities of ^{14}C detected as volatilized ^{14}C-organics (during headspace sampling), $^{14}CO_2$, solvent-extracted organics, and combusted $^{14}CO_2$ were summed for each flask.

The mass balances for flasks that were incubated and sampled for 26 weeks were conducted as follows. Radioactivity levels determined during each periodic sampling were recorded, and spreadsheets indicating cumulative percent ^{14}C and $^{14}CO_2$ evolved were maintained. At the termination of a given experiment, test flasks were acidified with 10 milliliters of 1 N HCl, hooked to the volatile sampling apparatus, and purged for 30 minutes to sample the entire volume of headspace. The radioactivity obtained in these samplings was recorded on the spreadsheets, and the total amount of $^{14}CO_2$ evolved over the duration of the experiment was calculated. Test soils were

subsequently subjected to solvent extraction, and the concentrated extracts were analyzed for ^{14}C-parent compound and ^{14}C-organic biotransformation products by GC/MS-GFPC. A sample of the post-extraction soil from each test flask was combusted in a Lindberg combustion furnace. The radioactivity recovered as $^{14}CO_2$ from the combustion was determined by liquid scintillation counting. The total amount of radioactivity remaining in the soil after extraction was calculated, and this value was incorporated into the mass balance for each test flask. The mass balance of ^{14}C-test compound in each test flask was calculated by summing the radioactivity detected during periodic samplings, the final GFPC analysis, and the post-extraction combustion. For determining the mass balance, the results of the GC/MS-GFPC analyses were corrected for extraction efficiency (see Appendix A). Total percent test compound recovery was calculated by dividing the total recovered activity by the activity initially added to each flask.

3.5 ENHANCED MINERALIZATION STUDIES

3.5.1 Mineralization of Isodrin Under Composting Conditions

An experiment was conducted to evaluate the mineralization of ^{14}C-isodrin under the environmental conditions found in a compost pile. Previous research demonstrated that organic compounds normally considered to be resistant to microbial degradation may be degraded in the environmental conditions found in compost piles (Williams et al., 1988).

Compost was prepared by combining 170 grams of horse manure/straw mixture, 90 grams of RMA soil no. 3224, 70 grams of horse feed (Purina Balanced Blend 14), and 60 grams of distilled water in a stainless steel paint can. The compost was manually mixed for 15 minutes until it appeared homogeneous. The compost was stored in the sealed paint can at 4°C until the experiment was started.

Approximately 100 grams of compost was placed into each of two 500-ml glass jars with band-style screw caps and butyl rubber septa. Two holes were punched through the septa, and inlet and outlet gas ports of glass tubing were installed as in the non-volatile mineralization test flasks (see Figure 3-1). Two feet of 1/4-inch (interior diameter) Tygon tubing was attached to the inlet ports on the test vessels. The other end of the tubing was attached to CO_2 scrubbing traps that contained 100 milliliters of 10 N KOH.

Two feet of tygon tubing was used to connect the outlet ports on the test vessels to a series of gas sampling traps as used in the volatile compound mineralization studies (see Figure 3-4). A negative pressure air pump was used to pull CO_2-free air through the test vessels. The pump was automatically turned on for 30 minutes at a time, six times per day, by an

electronic timer. The air flow rate was 33 milliliters per minute, or 1 liter per 30-minute sampling period. Thus, the gas volume of the test flasks (500 ml) was replaced twelve times per day.

The first and fourth gas trapping vials in the series were left empty to trap condensate and backflow from the other trapping vials. The second and third vials each contained 10 milliliters of acidified Betafluor LSC cocktail to trap volatilized organic compounds but to allow CO_2 to pass through. Betafluor was acidified with 25 milliliters of concentrated HCl per liter of Betafluor. The fifth and sixth traps each contained 10 milliliters of 2 N KOH to trap $^{14}CO_2$ produced from ^{14}C-isodrin mineralization.

The compost-containing test vessels were placed inside an incubator that was maintained at 54 $\pm$ 2°C during the test period. This temperature is representative of a full-scale thermophilic compost pile and was proven effective in degrading explosive compounds, such as trinitrotoluene, in a full-scale composting study (Williams et al., 1988). The tubing that connected the test vessels to the CO_2-scrubbing and gas sampling vessels was run out a hole in the side of the incubator, and these scrubbing vessels were maintained at ambient laboratory temperature (22 $\pm$ 2°C).

The experiment was initiated by injecting 200,000 dpm (0.1 uCi) of ^{14}C-isodrin into each of the two test vessels, mixing the compost, and re-sealing the caps. The headspace in each flask was automatically sampled as described previously, and the fluid contained in the sampling vials was changed and analyzed for ^{14}C activity twice per week.

The acidified Betafluor gas sampling traps were removed from the sampling train and replaced with fresh traps twice weekly. The used traps were capped with LSC vial caps, and the vials were analyzed for ^{14}C activity by LSC. The vials were stored overnight in the dark at 4°C before counting to minimize photochemiluminescence. Three background vials that contained 10 milliliters of acidified Betafluor were counted with each batch of samples, and the average activity detected in these background samples was automatically subtracted from subsequent sample vials.

The 2 N KOH gas sampling traps were removed from the sampling train and replaced with fresh traps twice weekly. Triplicate 1-ml samples of KOH from each trap were transferred to LSC vials that contained 20 milliliters of Aquasol-2 LSC cocktail. The vials were stored overnight in the dark at 4°C before counting to minimize photochemiluminescence. Three background vials that contained 20 milliliters of Aquasol-2 and 1 milliliter of 2 N KOH were counted with each batch of samples, and the average activity detected in these background samples was automatically subtracted from subsequent sample vials.

Condensate that collected in the first gas sampling vial was sampled and analyzed for ^{14}C activity on day 39 of the study. The condensate was sampled and counted in the same manner as the KOH gas sampling traps. The experiment was terminated after 39 days.

SECTION 4

RESULTS

4.1 CHEMICAL/PHYSICAL SOIL PROPERTIES

The results of WESTON's soil analyses indicated that the RMA test soils had variable physical and chemical properties (Table 4-1).

4.2 BIOLOGICAL SOIL PROPERTIES

The ability to mineralize a mixture of ^{14}C-glucose, ^{14}C-acetate, and ^{14}C-aminio acids was used to determine the metabolic capability of the soils received from RMA. Uncontaminated RMA soil, as well as soils from borehole nos. 3215, 3224, and 3256, exhibited heterotrophic microbial activity and were used in mineralization studies, while soils from borehole nos. 3204 and 3219 showed no metabolic activity and were not used in mineralization studies (Table 4-2, Figures 4-1 through 4-6). Error bars indicating ± one standard deviation are included on all metabolic potential figures (Figures 4-1 through 4-6). For data points where error bars are not visible, the 95-percent confidence interval was too small to represent graphically.

4.3 CONTAMINANT CONCENTRATIONS IN RMA SOILS

The concentrations of DIMP, isodrin, dieldrin, DBCP, and PCPMSO in the RMA test soils as determined by WESTON are presented in Table 4-3. The contaminant data for soils as provided to WESTON from analyses conducted by ESE and Ebasco are presented in Appendix B.

4.4 MINERALIZATION STUDIES

Bar charts indicating the mass balances of ^{14}C-test compounds are presented in this section. Three compartments are represented for isodrin (no volatilized ^{14}C-isodrin was quantified), and four compartments are presented for dieldrin, DIMP, DBCP, and PCPMSO. The compartments are: 1) $^{14}CO_2$ -- percent of the ^{14}C-spike that mineralized during the study, 2) ^{14}C-organics -- percent of the ^{14}C-spike that was detected as volatilized organic compounds during the study, 3) extracted -- percent of the ^{14}C-spike that was extracted from soil and detected as parent compound after termination of the test flask(s), 4) nonextractable -- percent of the ^{14}C-spike that was detected as $^{14}CO_2$ by combusting post-extraction soils.

Table 4-1

Chemical and Physical Properties of RMA Soils

Parameter	Soil No. 3224	Soil No. 3256	Soil No. 3215	Soil No. 3204	Soil No. 3219	Uncontaminated Soil
Soil pH	8.1	9.4	8.2	11.2	6.5	7.8
TOC (%)	0.63	0.22	0.21	1.81	4.03	0.12
Soil moisture (%)	29.1	14.2	10.0	42.7	21.6	10.9
Water holding capacity (g/g)	0.52	0.44	0.19	0.96	1.29	0.51
Total kjeldahl nitrogen (mg/kg)	374	278	47	577	726	543
Total phosphorus (mg/kg)	770	627	380	1,640	2,560	336

Table 4-2

Biological Properties of RMA Soils

Parameter	Soil No. 3224	Soil No. 3256	Soil No. 3215	Soil No. 3204	Soil No. 3219	Uncontaminated Soil
Test compound present in soil	isodrin dieldrin PCPMSO	DIMP	DBCP			None
Total heterotrophs (CFU per g soil)[a]	1.6×10^4	2.1×10^3	3.5×10^4	0	1.4×10^3	2.7×10^6
Mean 21-day metabolic potential (%)[b]	26.3	17.6	28.4	0.1	0.1	24.5
^{14}C-MPN (CFU per g soil)	8.0×10^2	$<10^2$			$<10^2$	7.9×10^4

[a]Nutrient agar plates.
[b]Percent of ^{14}C-glucose, ^{14}C-amino acids, ^{14}C-acetate mixture mineralized to $^{14}CO_2$ compared to amount originally added as parent compounds to each flask.

Figure 4-1. Plot of percent ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate mineralized in RMA soil #3204.

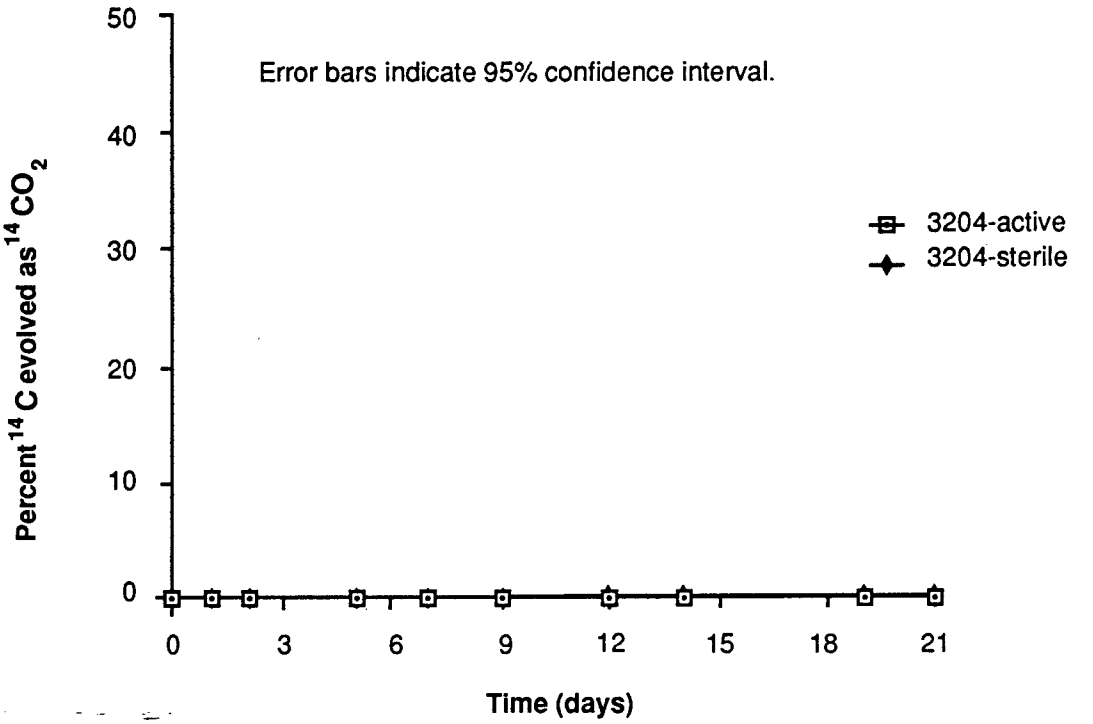

Figure 4-2. Plot of percent ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate mineralized in RMA soil #3215.

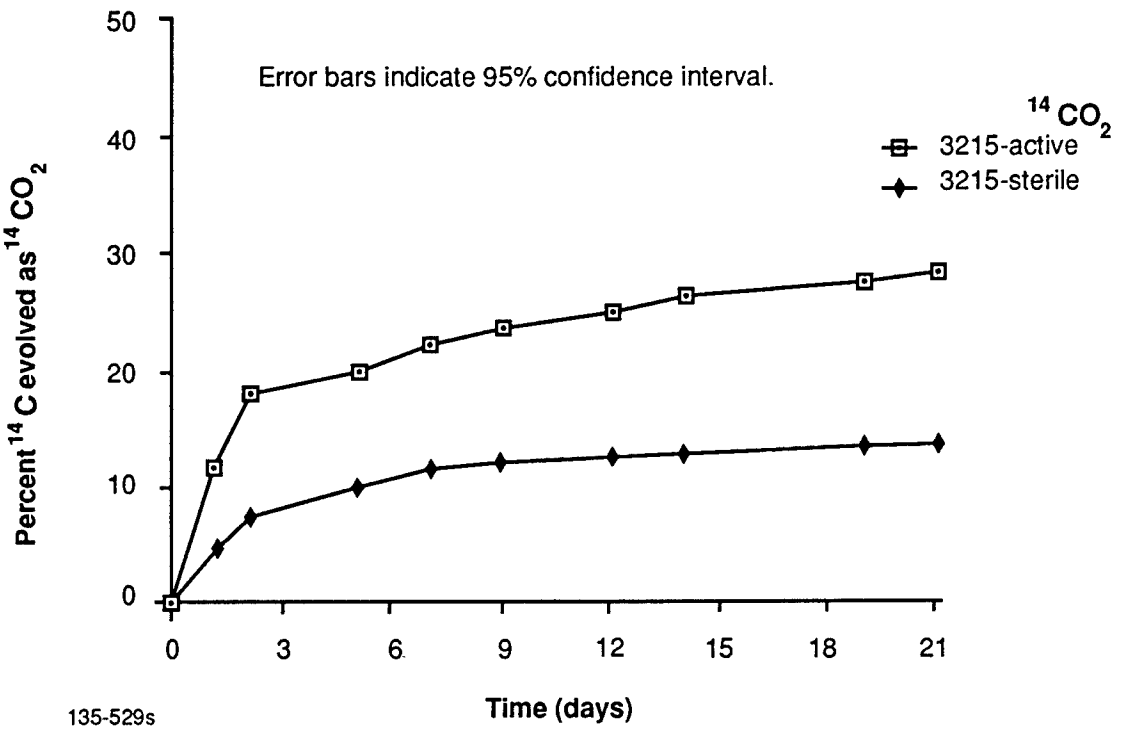

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Figure 4-3. Plot of percent ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate mineralized in RMA soil #3219.

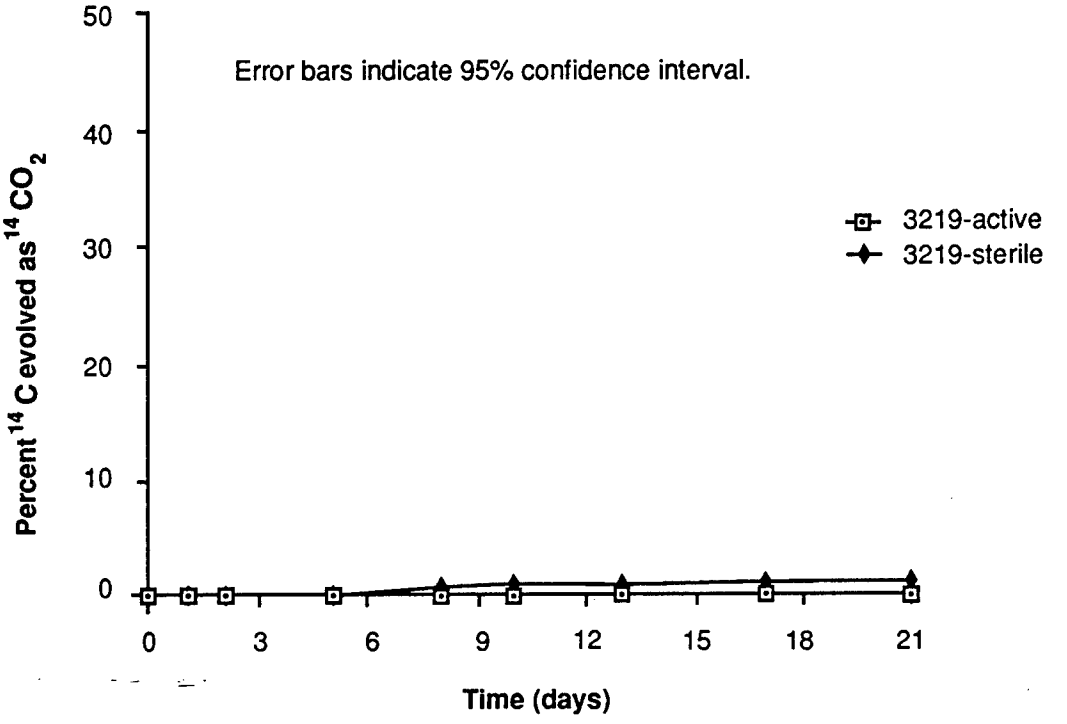

Figure 4-4. Plot of percent ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate mineralized in RMA soil #3224.

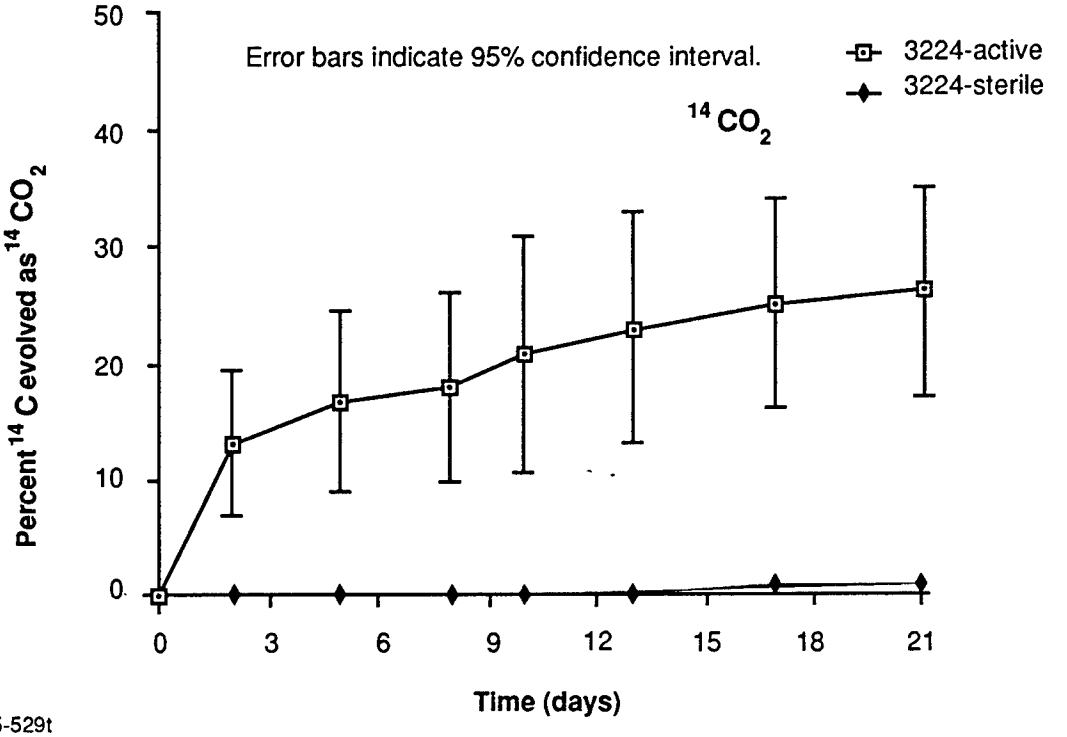

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Figure 4-5. Plot of percent ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate mineralized in RMA soil #3256.

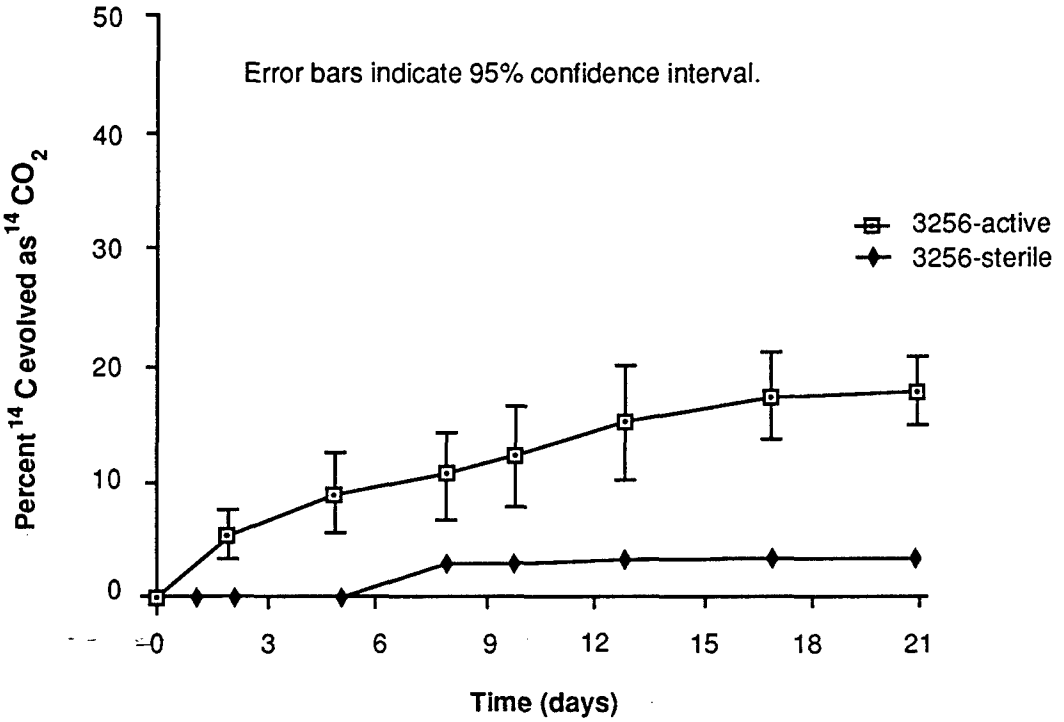

Figure 4-6. Plot of percent ^{14}C-glucose, ^{14}C-amino acids, and ^{14}C-acetate mineralized in RMA uncontaminated soil.

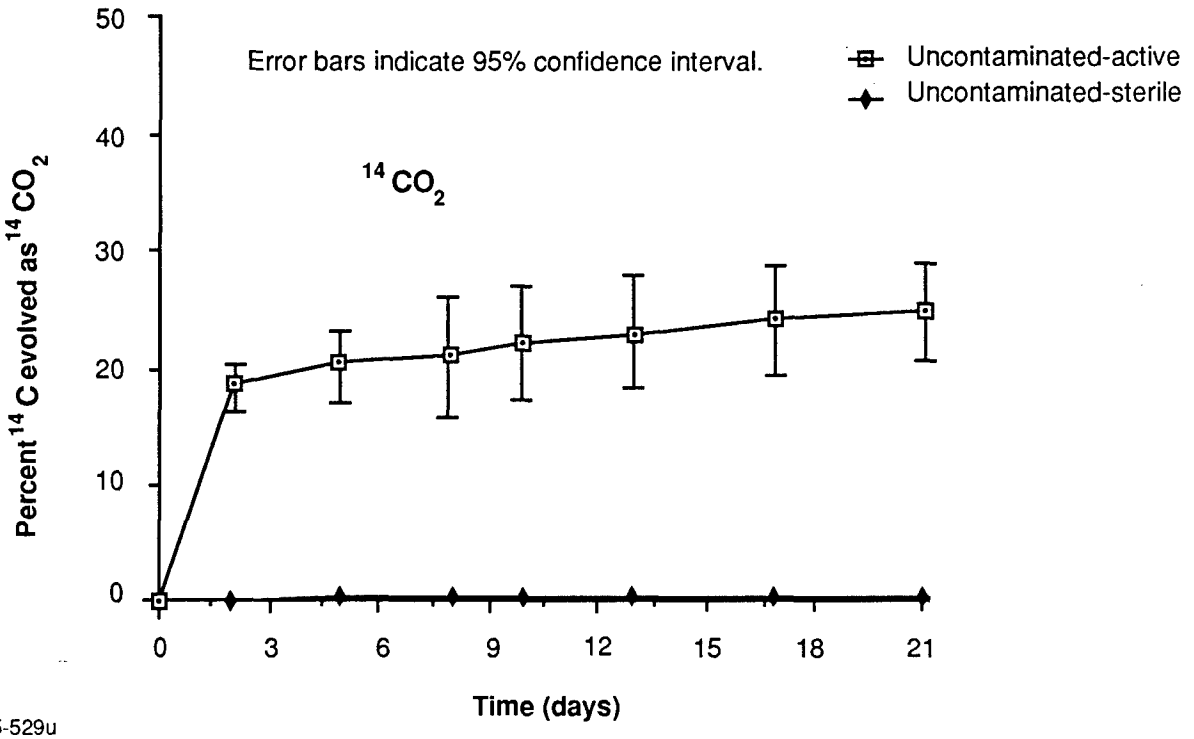

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Table 4-3

Concentrations of DIMP, Dieldrin, Isodrin, DBCP, and PCPMSO in RMA Soils

Contaminant (mg/kg)	Soil No. 3224	Soil No. 3256	Soil No. 3215	Soil No. 3204	Soil No. 3219	Uncontaminated Soil
DIMP	0.24	0.14	<0.05	<0.07	0.22	<0.05
Dieldrin	3.1	ND	ND	41	1.4	ND
Isodrin	51.0	ND	ND	7.5	10.0	ND
DBCP	<0.005	<0.006	<0.005	<0.009	<0.005	<0.006
PCPMSO	<500	<0.5	<0.5	<100	<100	<0.5

ND - Not detected.

4.4.1 Dieldrin

After 182 days of incubation, dieldrin was mineralized to the extent of 4.6 and 3.4 percent in RMA uncontaminated and no. 3224 soils, respectively (see Figures 4-7 and 4-9). One of the soil no. 3224 flasks was broken 126 days into the test period and was not replaced because of the low levels of mineralization observed in the flask up to that time. Consequently, 95-percent confidence intervals could not be calculated for soil no. 3224 subsequent to day 126 of the study.

A comparison of the 95-percent confidence interval ranges indicated that after 112 days of incubation, the mean percent mineralization of dieldrin in active RMA uncontaminated soil was significantly greater than that observed in sterile RMA uncontaminated soil at the 95-percent confidence level. Comparisons of the confidence intervals of 1) active versus sterile soil no. 3224 and 2) active uncontaminated versus active soil no. 3224 indicated no statistically significant difference in the extent of dieldrin mineralization between these soil types.

Mass balances of ^{14}C-dieldrin in time-zero and 26-week test flasks indicated that the majority of the ^{14}C-dieldrin remained as undegraded test compound in all test flasks (see Figures 4-8 and 4-10). Total recovery of ^{14}C activity ranged from 43 to 117 percent in time-zero flasks and from 54 to 109 percent in 26-week test flasks.

4.4.2 DIMP

After 183 days of incubation, DIMP was mineralized to the extent of 6.4 and 6.0 percent in RMA uncontaminated and no. 3256 soils, respectively (see Figures 4-11 and 4-13). At the 95-percent confidence level, no statistically significant difference in the extent of DIMP mineralization was observed between any soil types over the test period.

Mass balances of ^{14}C-DIMP in time-zero and 26-week test flasks indicated that the majority of the ^{14}C-DIMP was recovered as undegraded parent compound in all test flasks. Total ^{14}C-activity recovery ranged from 45 to 92 percent in time-zero flasks and from 18 to 155 percent in 26-week flasks (see Figures 4-12 and 4-14).

4.4.3 Isodrin

After 193 days of incubation, ^{14}C-isodrin was mineralized to the extent of 1.5 and 0.4 percent in RMA uncontaminated and no. 3224 soils, respectively (see Figures 4-15 and 4-17).

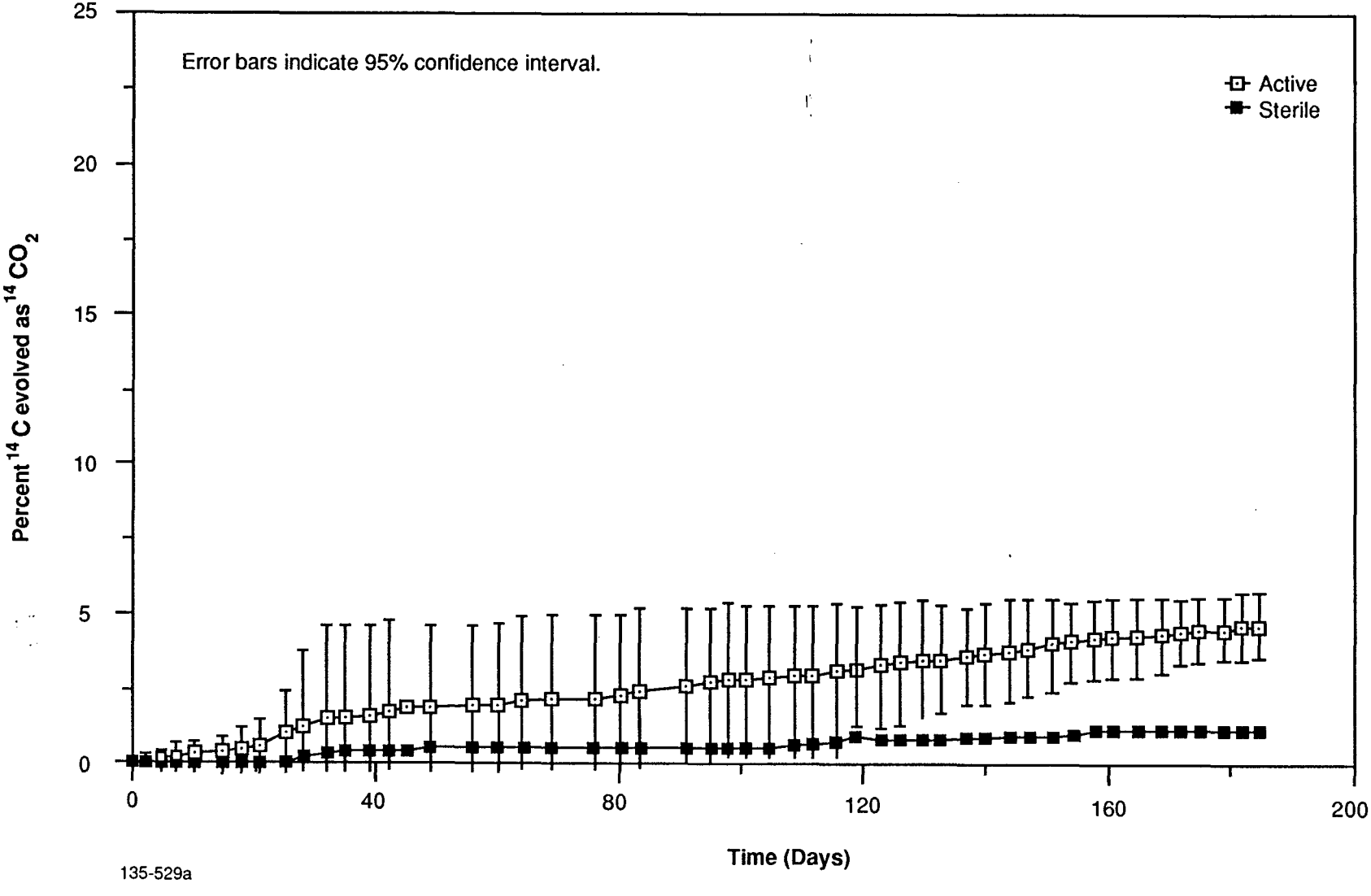


Figure 4-7. ^{14}C-dieldrin evolved as $^{14}CO_2$ in RMA uncontaminated soil.

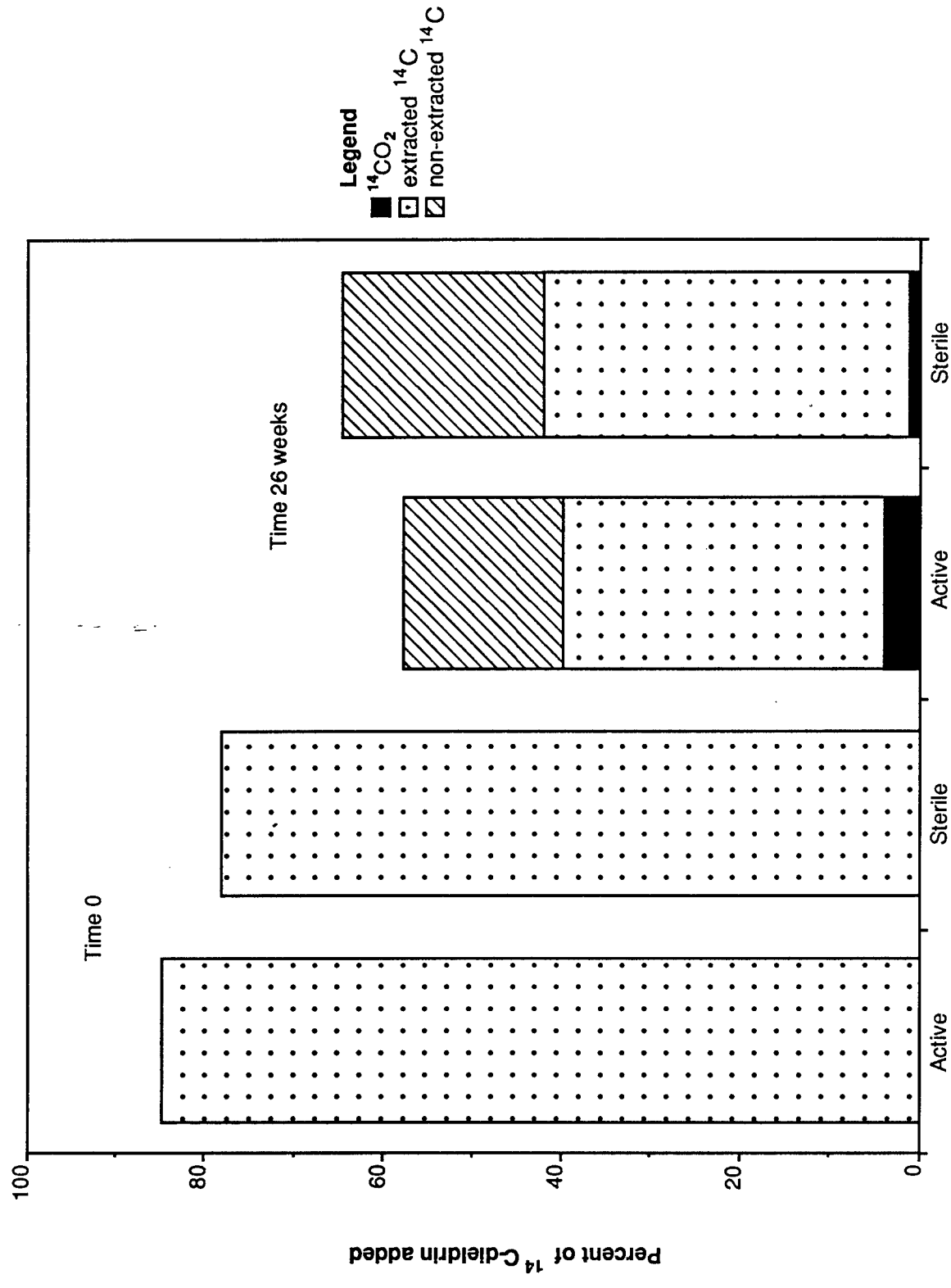


Figure 4-8. Mass balance of ^{14}C-dieldrin in RMA uncontaminated soil.

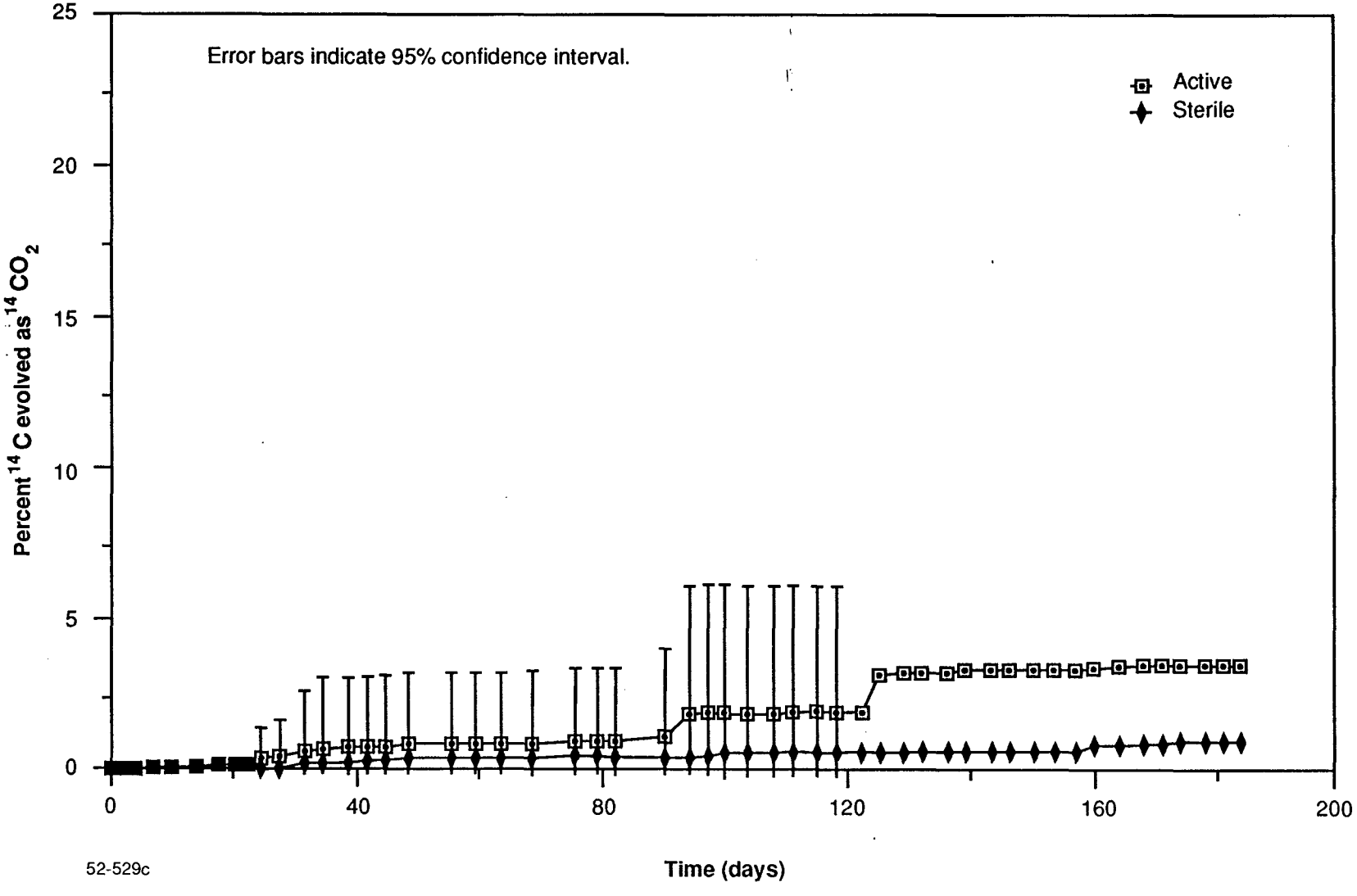
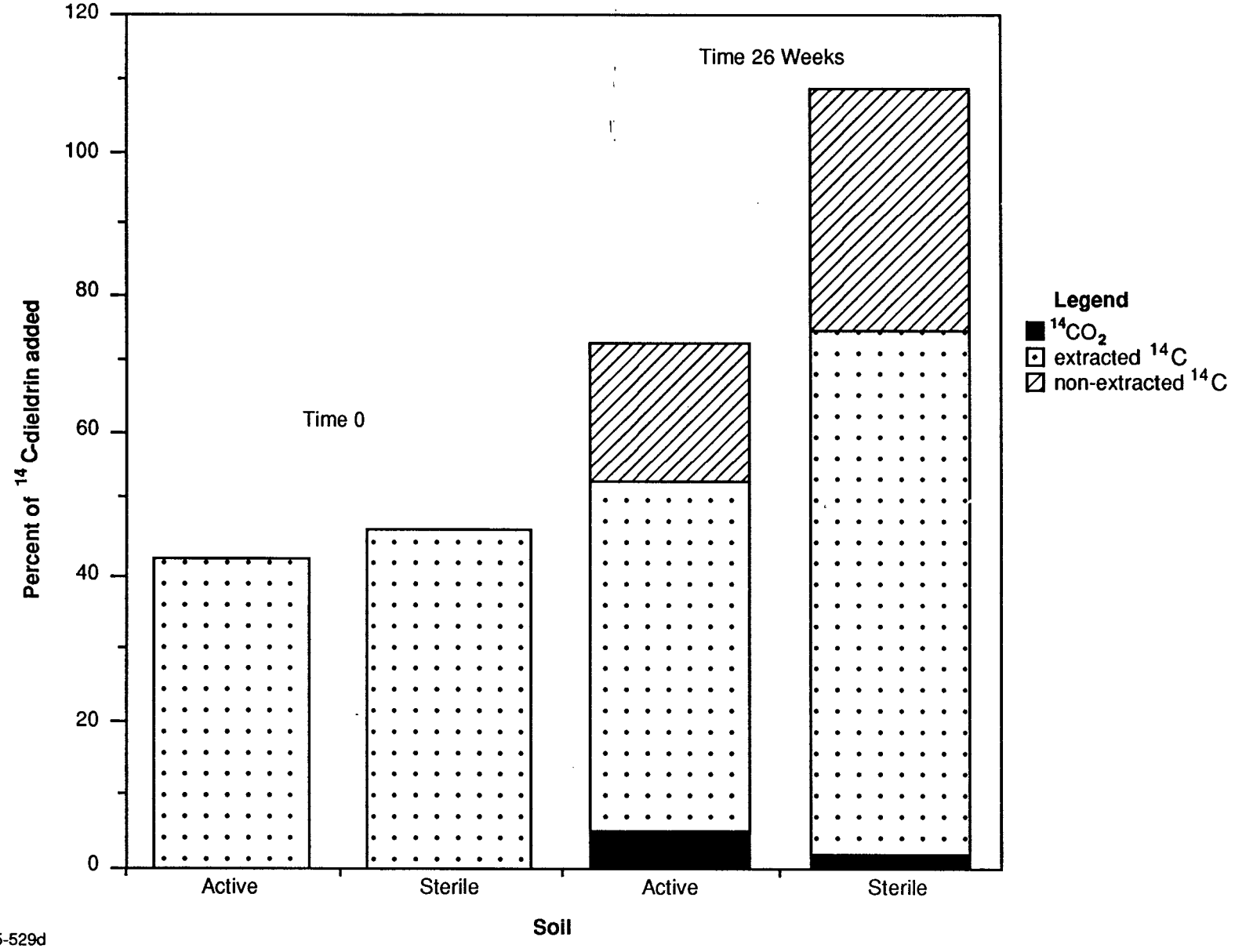


Figure 4-9. ^{14}C-dieldrin evolved as $^{14}CO_2$ in RMA 3224 soil.



135-529d

Figure 4-10. Mass balance of ^{14}C-dieldrin in RMA 3224 soil.

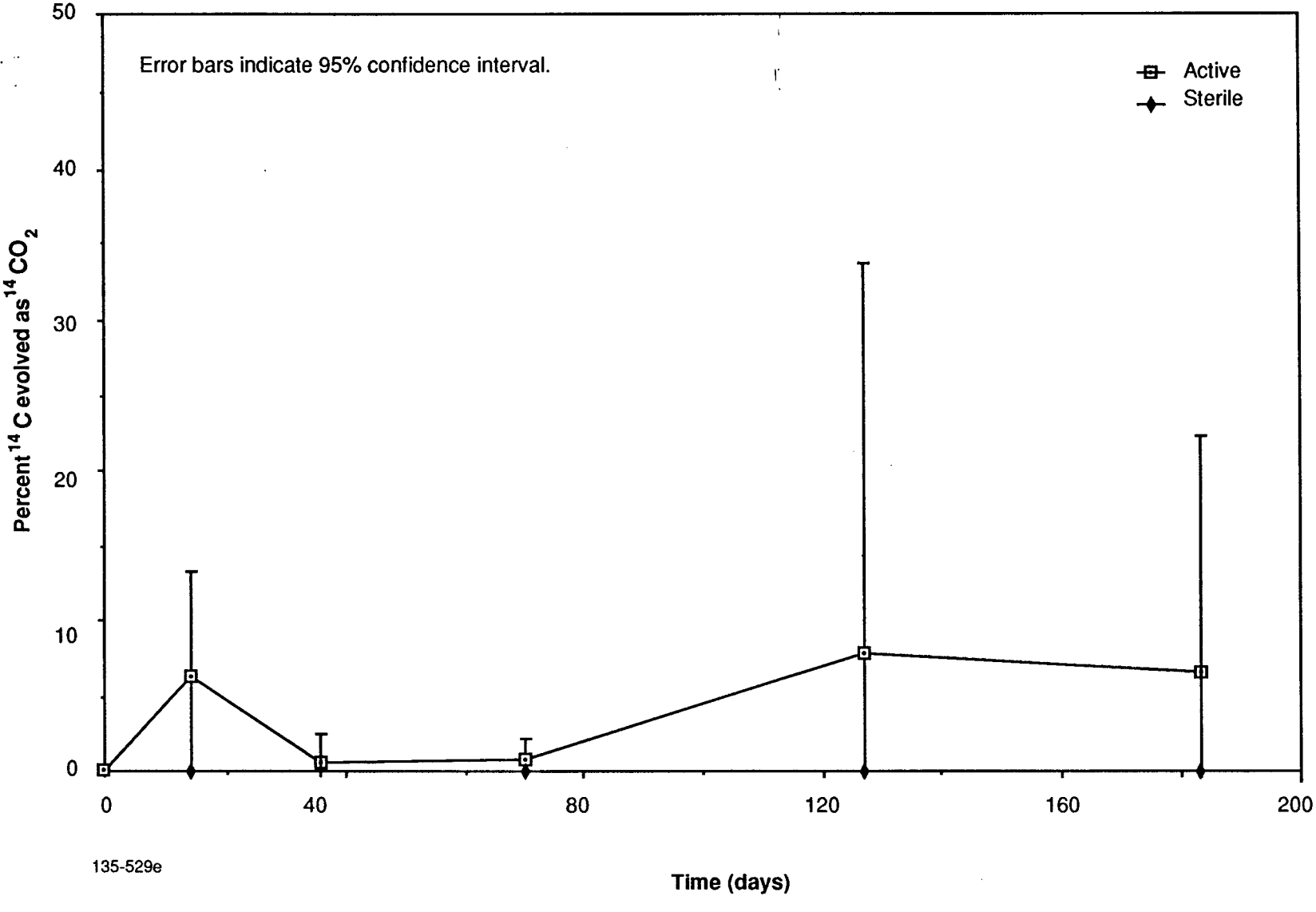


Figure 4-11. ^{14}C-DIMP evolved as $^{14}CO_2$ in RMA uncontaminated soil.

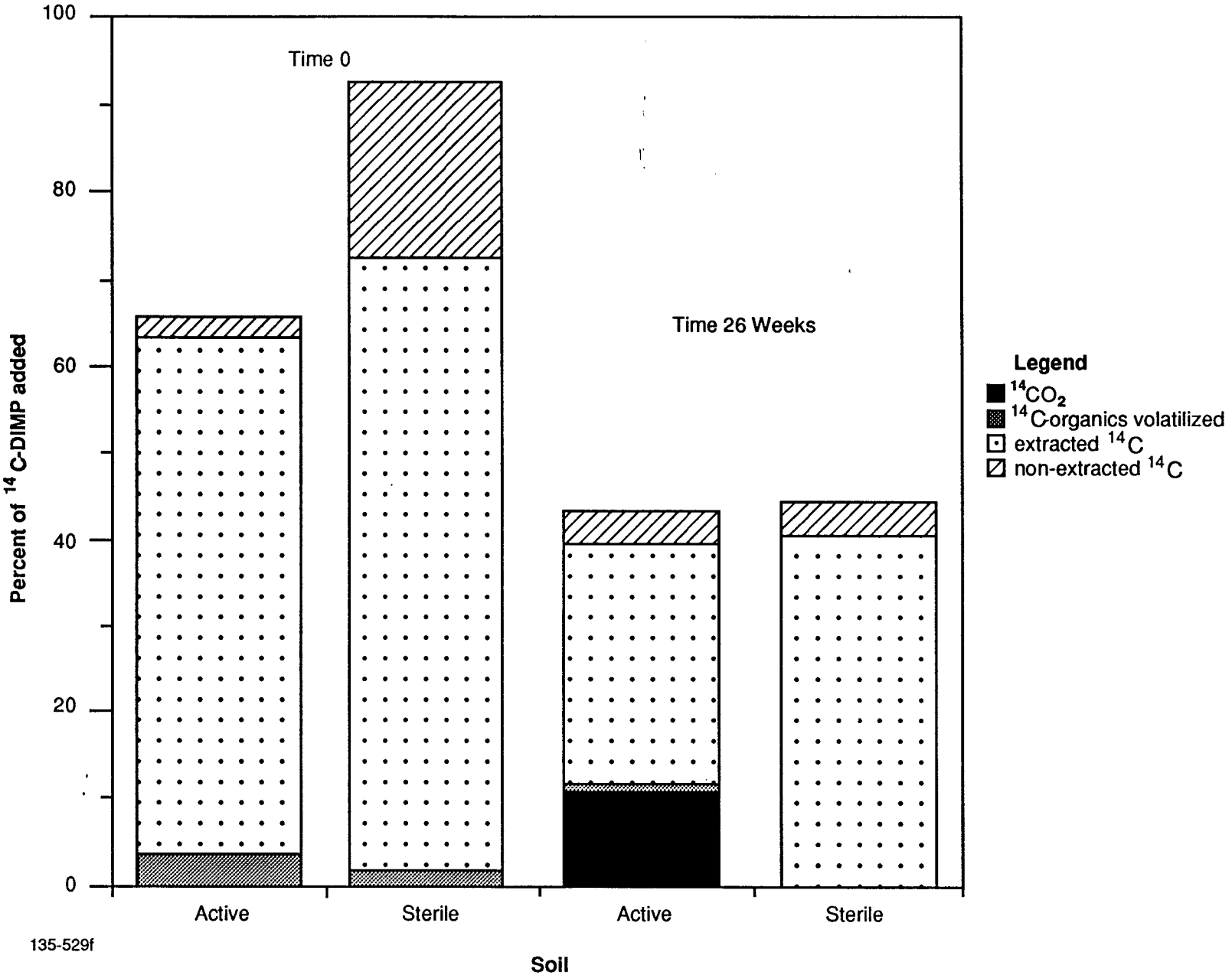


Figure 4-12. Mass balance of ^{14}C-DIMP in RMA uncontaminated soil.

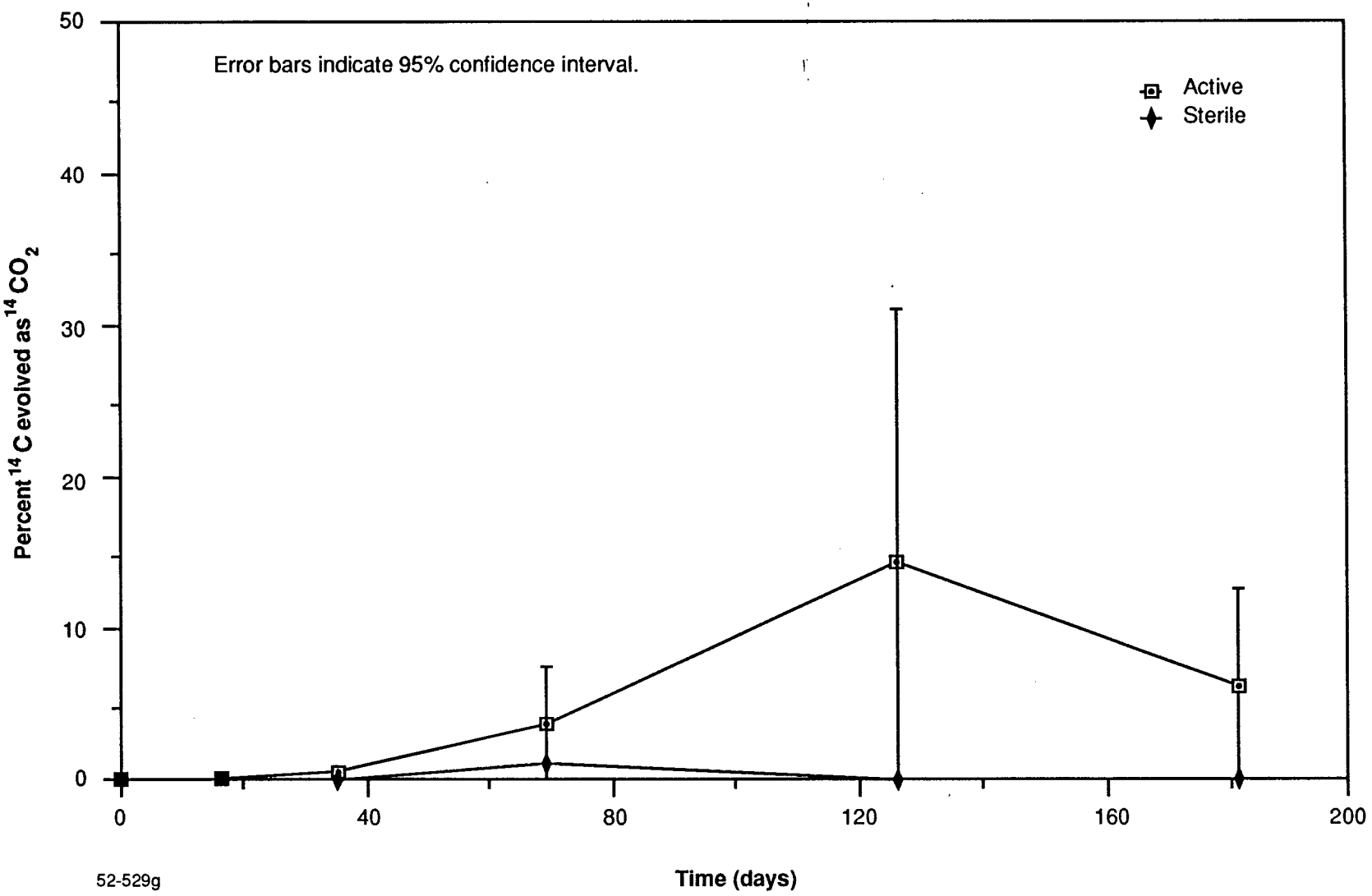


Figure 4-13. ^{14}C-DIMP evolved as $^{14}CO_2$ in RMA 3256 soil.

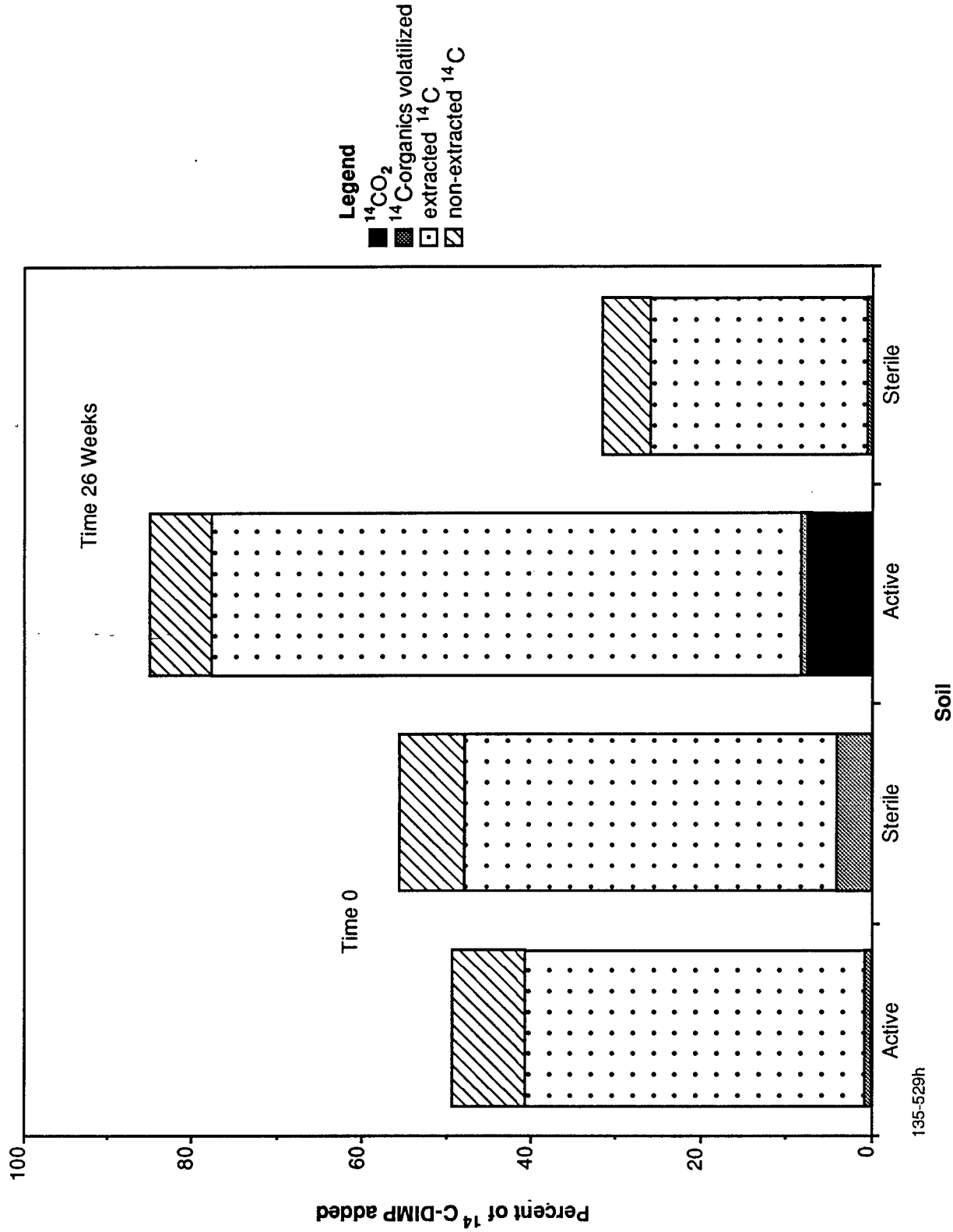


Figure 4-14. Mass balance of ^{14}C-DIMP in RMA 3256 soil.

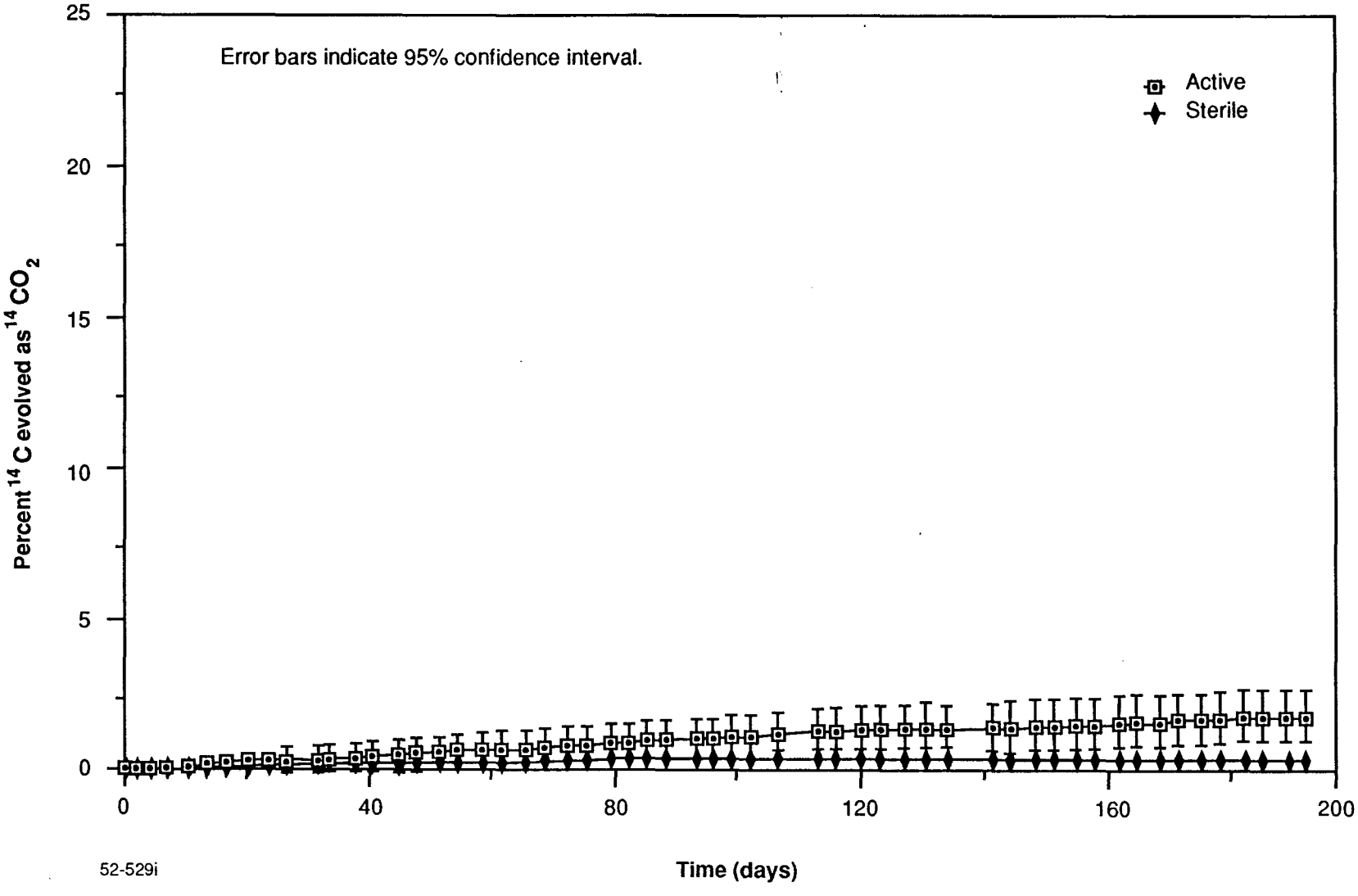


Figure 4-15. ^{14}C-Isodrin evolved as $^{14}CO_2$ in RMA uncontaminated soil.

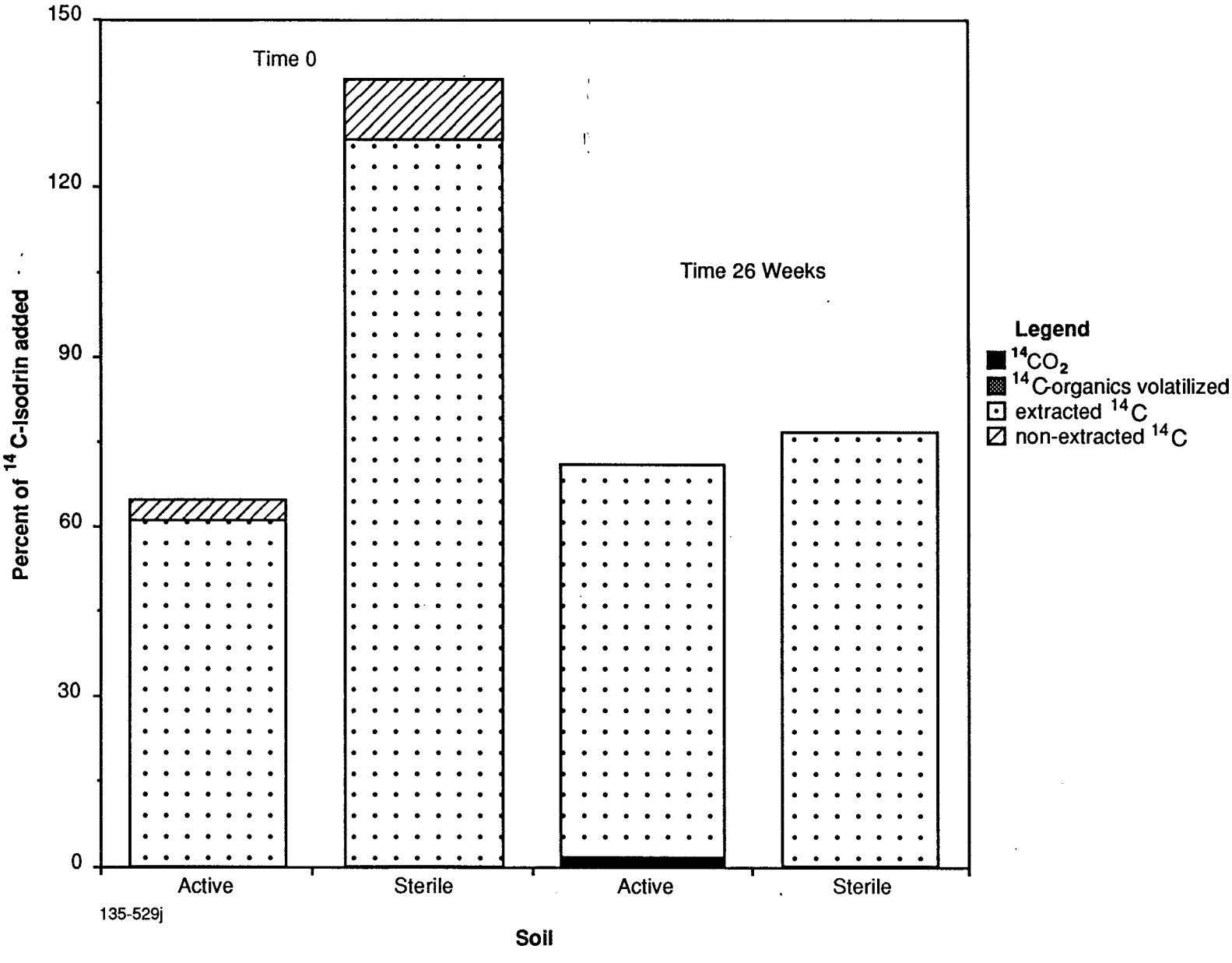


Figure 4-16. Mass balance of ^{14}C-Isodrin in RMA uncontaminated soil.

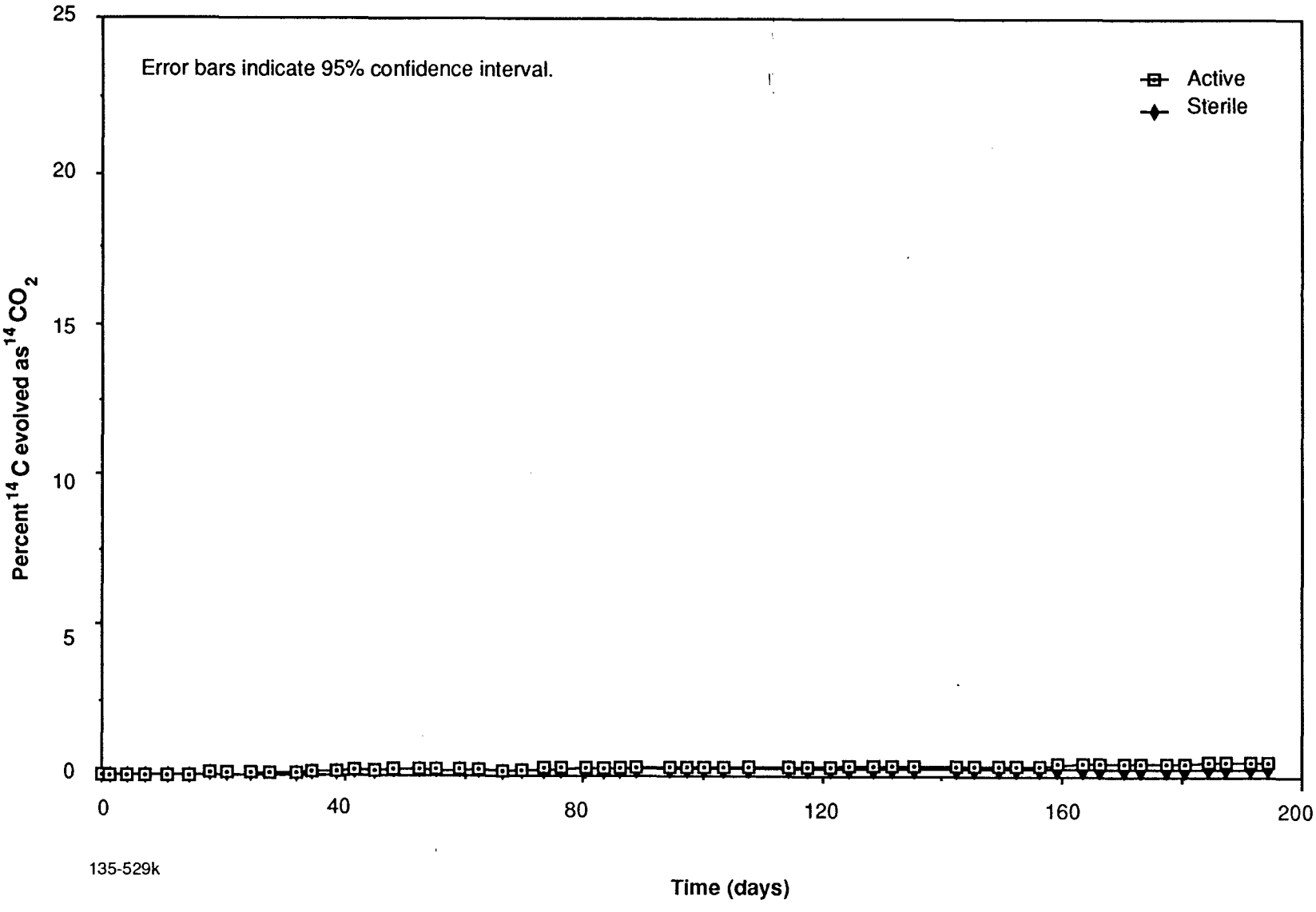


Figure 4-17. ^{14}C-Isodrin evolved as $^{14}CO_2$ in RMA 3224 soil.

After day 90 of the study, the mean percent mineralization of isodrin in active RMA uncontaminated soil was significantly greater than that observed in sterile RMA uncontaminated soil at the 95-percent confidence level. After day 160 of the study, the mean percent mineralization of isodrin in active RMA uncontaminated soil was significantly greater than that observed in active RMA soil no. 3224 at the 95-percent confidence level. No statistical difference in the extent of isodrin mineralization was observed between active and sterile RMA soil no. 3224.

Mass balances of ^{14}C-isodrin indicated that the majority of the test compound was recovered as undegraded parent compound in the initial and final test flasks (see Figures 4-16 and 4-18). Total recovery of ^{14}C activity ranged from 40 to 137 percent in time-zero flasks and from 59 to 117 percent in 26-week test flasks. No ^{14}C transformation products were detected in the 26-week test flasks by GC/MS-GFPC.

4.4.3.1 Degradation of Isodrin Under Composting Conditions

The isodrin composting experiment was conducted for a period of 39 days. A total of less than 0.1 percent of the ^{14}C-isodrin added to each test vessel was recovered as ^{14}C activity in the Betafluor, KOH, and condensate traps.

4.4.4 DBCP

After 182 days of incubation, ^{14}C-DBCP was mineralized to the extent of 0.2 and 0.8 percent in RMA uncontaminated soil and soil no. 3224, respectively (see Figures 4-19 and 4-21). At the 95-percent confidence level, no statistical difference in the mineralization of ^{14}C-DBCP was observed among any soil types during the test period.

Mass balances of ^{14}C-DBCP indicated that the majority of the test compound was recovered as undegraded parent compound in all test flasks (see Figures 4-20 and 4-22). Total recovery of ^{14}C activity ranged from 22 to 105 percent in time-zero test flasks and from 19 to 39 percent in 26-week test flasks. No ^{14}C-transformation products were detected by GC/MS-GFPC.

4.4.5 PCPMSO

After 189 days of incubation, ^{14}C-PCPMSO was mineralized to the extent of 0.5 and 0.6 percent in RMA uncontaminated soil and soil no. 3215, respectively (see Figures 4-23 and 4-25). At the 95-percent confidence level, no statistical difference in ^{14}C-PCPMSO mineralization was observed in any soil types during the test period.

Mass balances of ^{14}C-PSPMSO indicated that a majority of the test compound was recovered as undegraded parent compound in all test flasks (see Figures 4-24 and 4-26). Total recovery of ^{14}C activity ranged from 95 to 126 percent in time-zero test flasks and from 43 to 90 percent in 26-week test flasks. No ^{14}C transformation products were detected by GC/MS-GFPC.

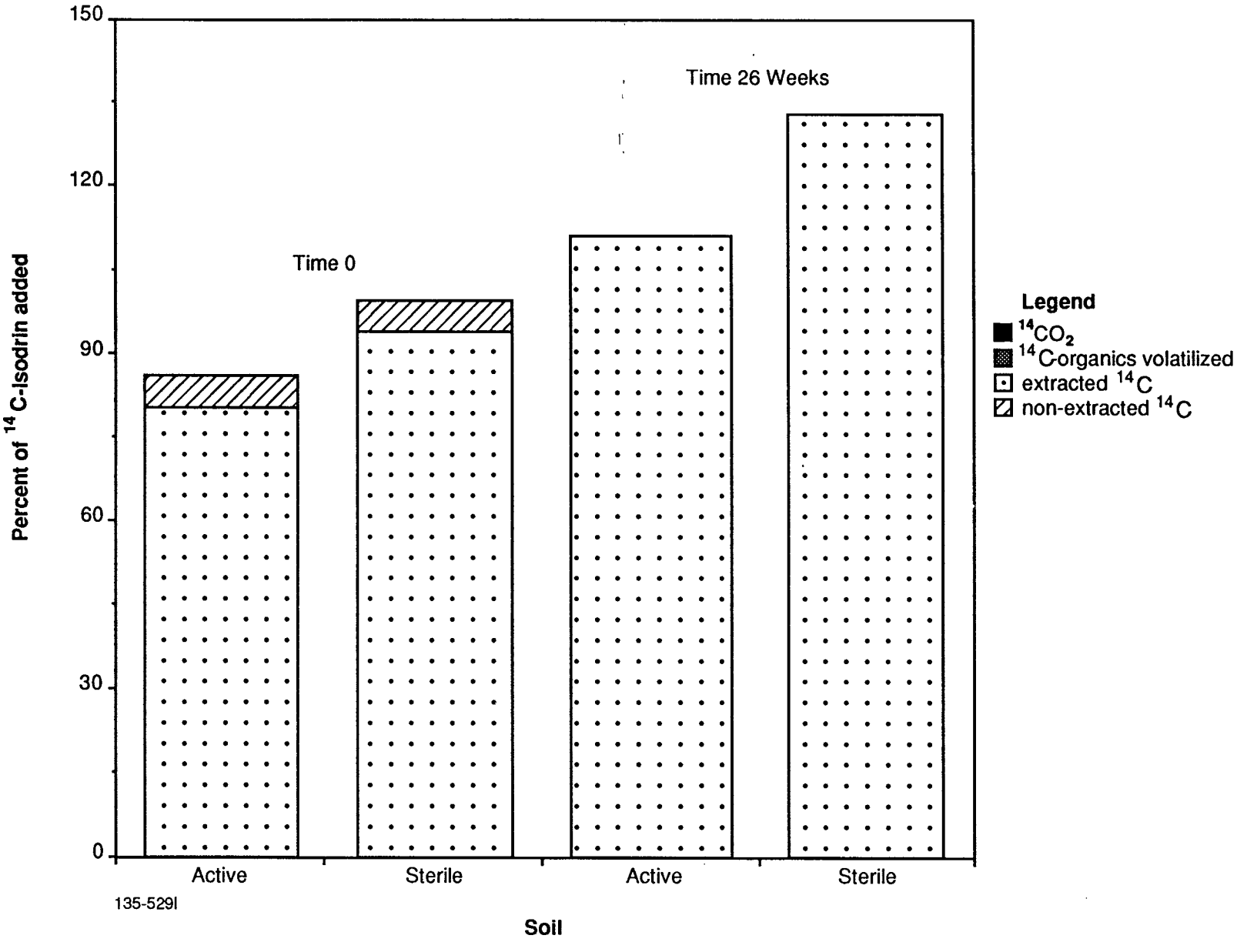


Figure 4-18. Mass balance of ^{14}C-Isodrin in RMA 3224 soil.

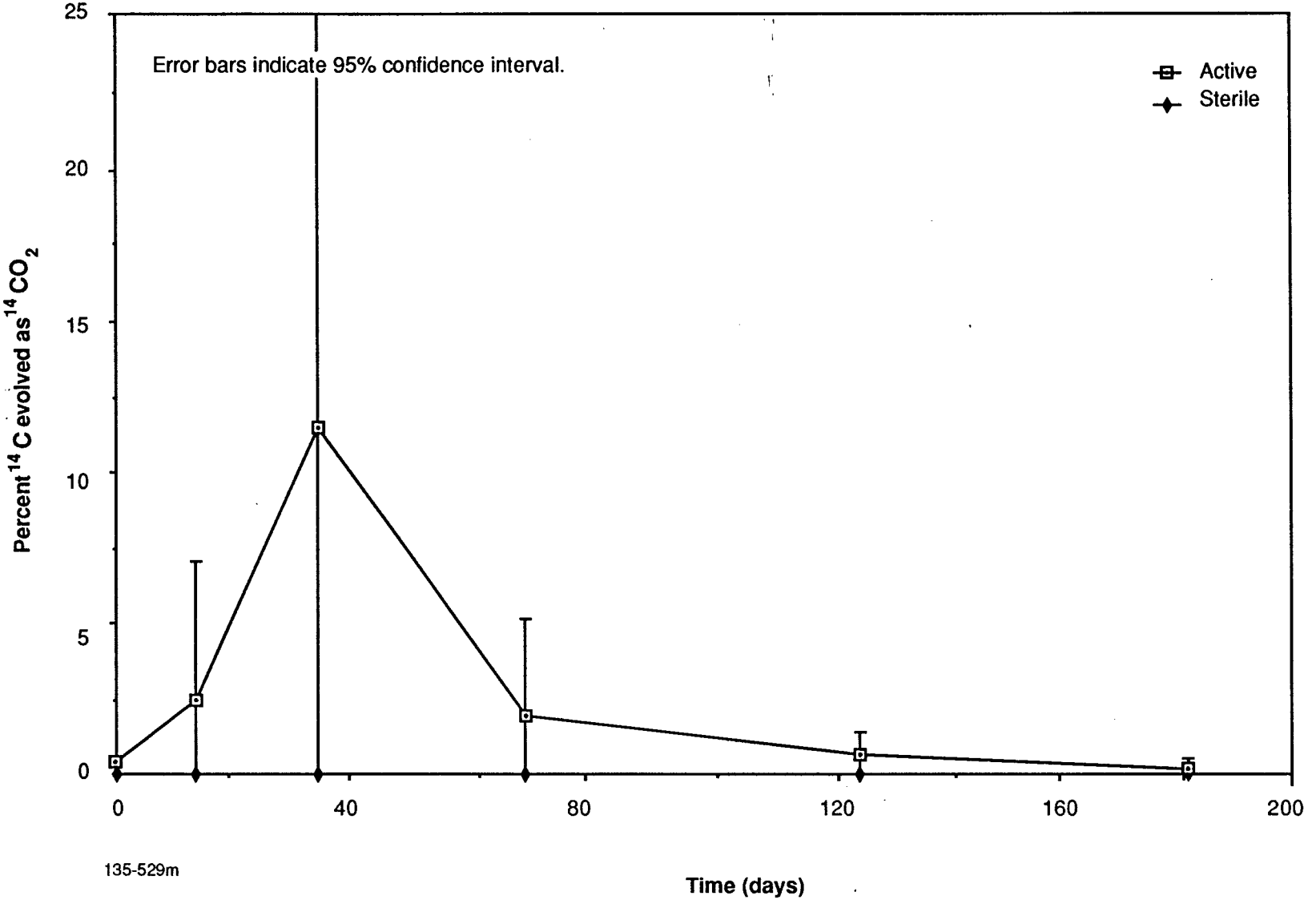


Figure 4-19. ^{14}C-DBCP evolved as $^{14}CO_2$ in RMA uncontaminated soil.

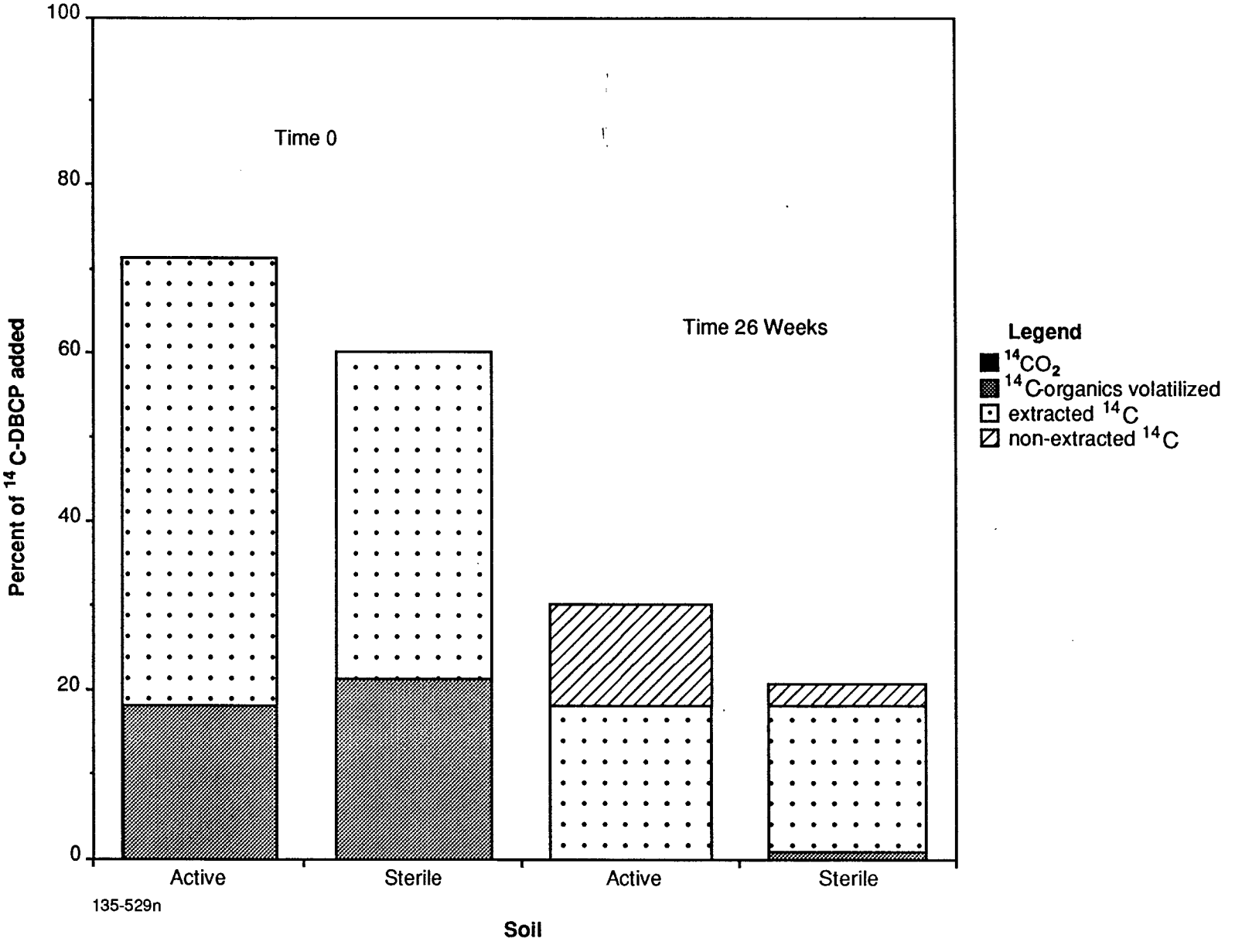


Figure 4-20. Mass balance of ^{14}C-DBCP in RMA uncontaminated soil.

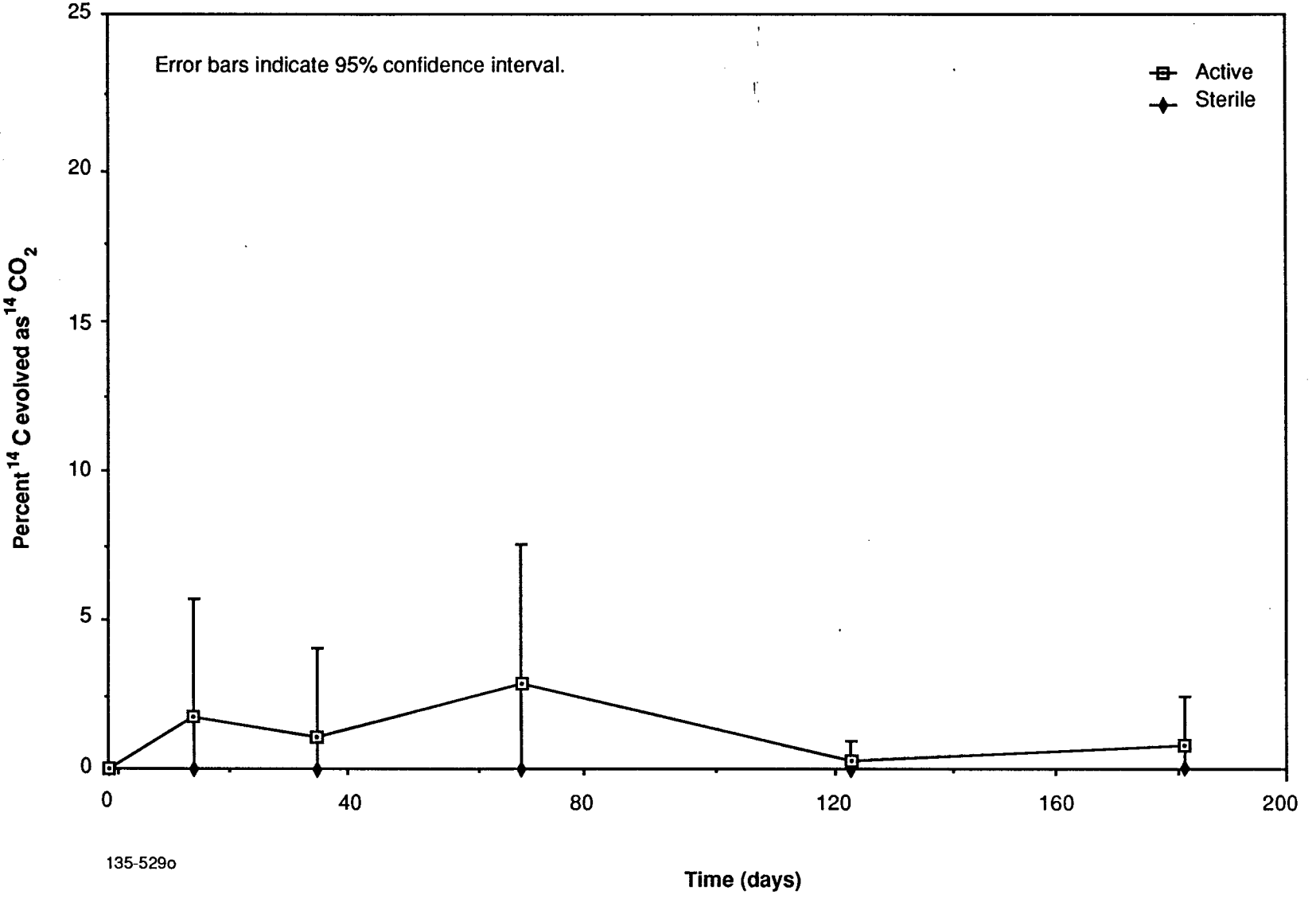


Figure 4-21. ^{14}C-DBCP evolved as $^{14}CO_2$ in RMA 3215 soil.

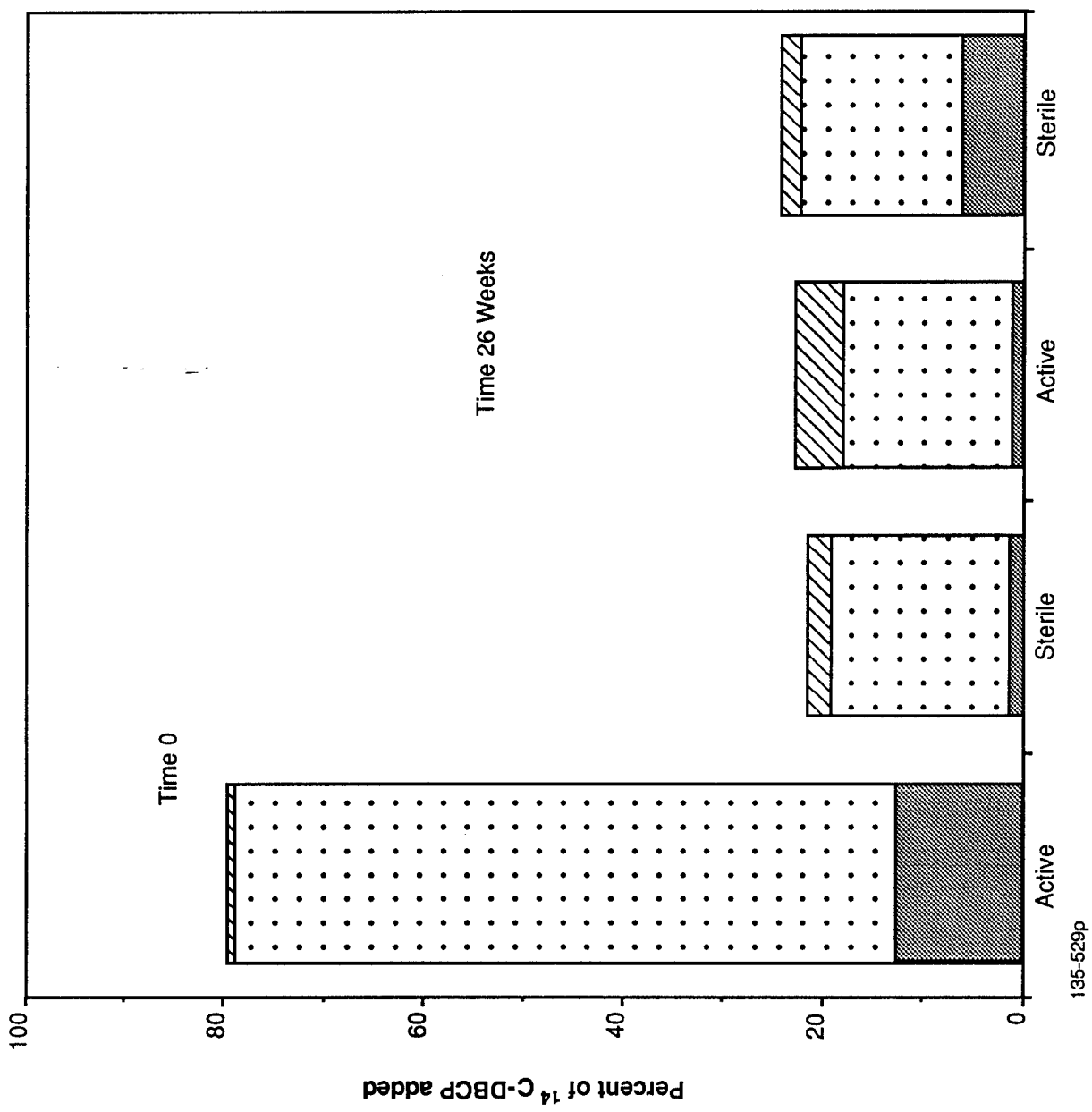


Figure 4-22. Mass balance of ^{14}C-DBCP in RMA 3215 soil.

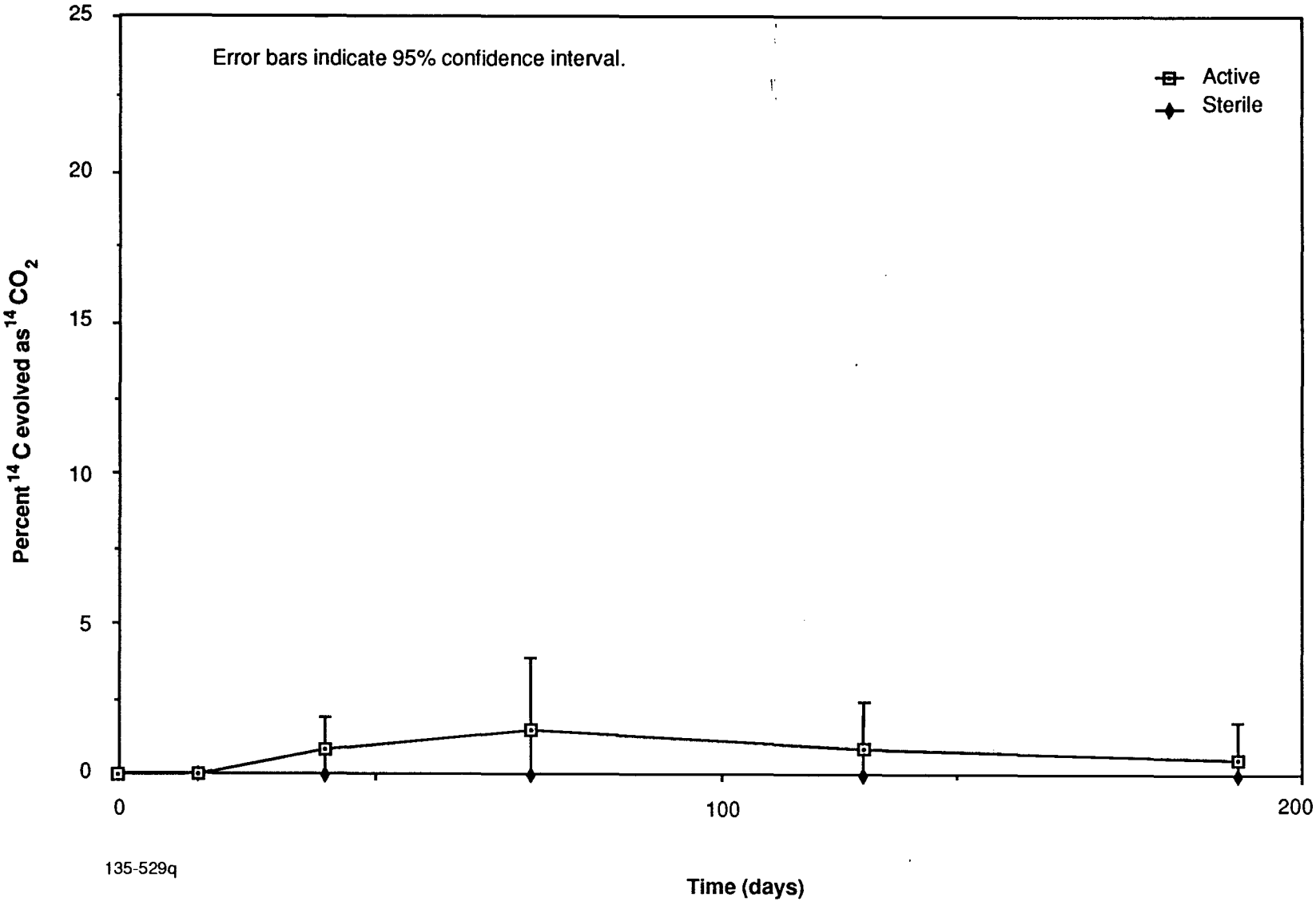


Figure 4-23. ^{14}C-PCPMSO evolved as $^{14}CO_2$ in RMA uncontaminated soil.

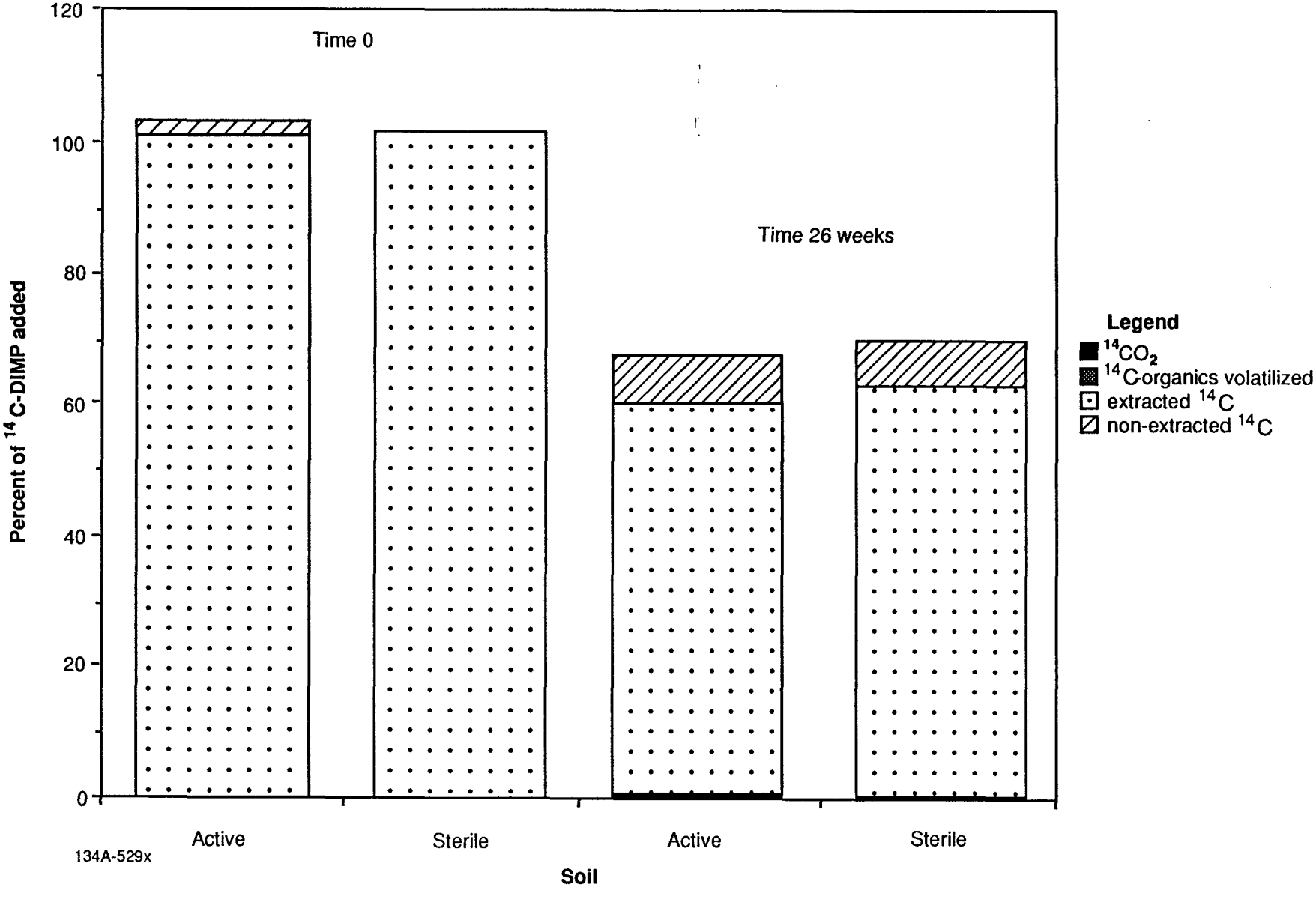


Figure 4-24. Mass balance of ^{14}C-PCPMSO in RMA uncontaminated soil.

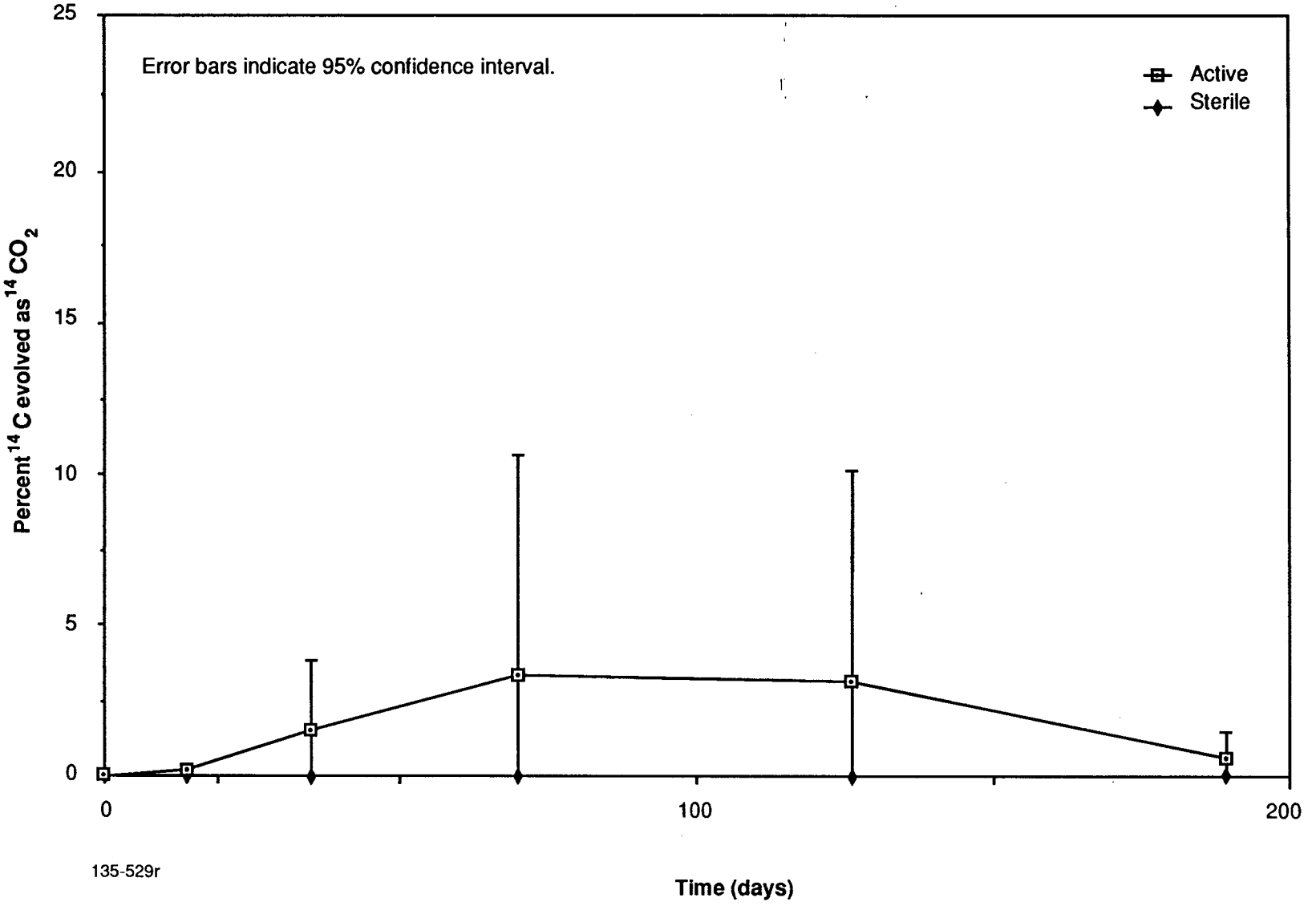


Figure 4-25. ^{14}C-PCPMSO evolved as $^{14}CO_2$ in RMA soil 3215.

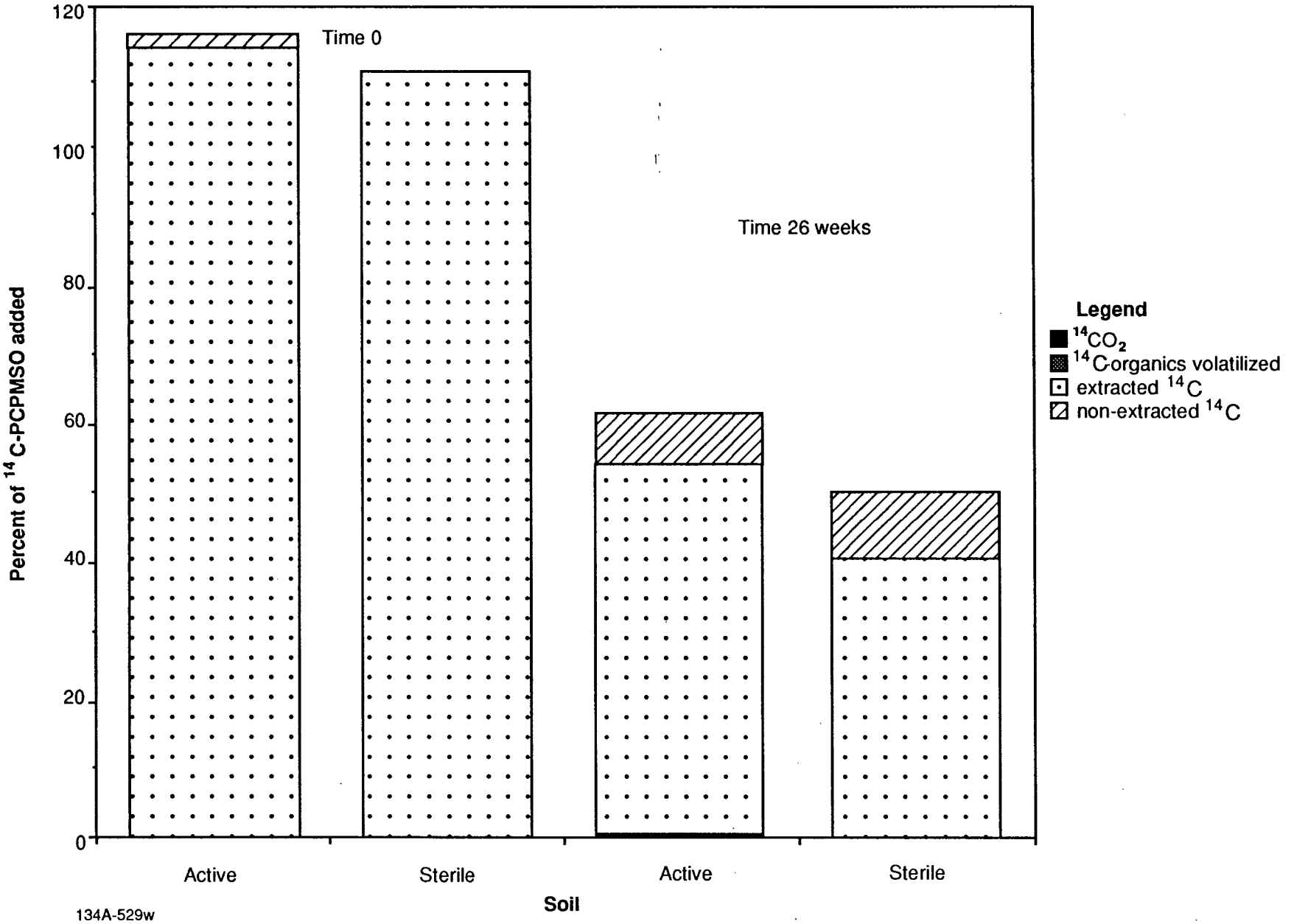


Figure 4-26. Mass balance of ^{14}C-PCPMSO in RMA soil #3215.

SECTION 5

DISCUSSION AND CONCLUSIONS

5.1 SOIL PROPERTIES

RMA soil nos. 3204 and 3219 exhibited little or no microbial activity (Table 4-2, Figures 4-1 and 4-3). Both of these soils appeared sandy but had TOC concentrations that were substantially higher than the other RMA soils, suggesting the presence of high concentrations of organic contaminants in these soils. Both soil nos. 3204 and 3219 contained substantial concentrations of organics and metals (see Appendix B).

Uncontaminated RMA soil contained a higher mean population of heterotrophic bacteria on the basis of plate counts than any of the contaminated RMA soils (see Table 4-2). This suggests that contaminants present in RMA soils inhibit microbial populations.

5.2 MINERALIZATION STUDIES

Data obtained in the present study suggest that inorganic and/or organic contaminants in RMA soils may inhibit in situ microbial metabolic activity. This statement is supported by several facts. Soil nos. 3204 and 3219 contained significantly higher concentrations of TOC than the other RMA soils, possibly indicative of high concentrations of organic contaminants. Soil nos. 3204 and 3219 exhibited little or no heterotrophic microbial activity. In addition, the extent of dieldrin and isodrin mineralization was slightly higher (but not statistically different) in uncontaminated versus contaminated soils. Adaptation to the test compounds in contaminated RMA soils, if it has occurred at all, appears to be insignificant and without consequence regarding transformation of the test compounds.

Beginning at 125 days into the dieldrin test period, the percent mineralization in sterilized RMA soil no. 3224 was less than the 95-percent confidence interval of the three triplicate flasks that contained active uncontaminated soil (Figure 4-7). This also was the case for the isodrin study beginning on day 87. These data indicate that microbial activity was responsible for test compound mineralization beyond that incurred by chemical processes.

Previously published literature indicates that at least some of the test compounds are microbially transformed to organic intermediates (see Subsection 2.1). However, no ^{14}C-transformation products were detected by GC/MS-GFPC in any of the mineralization studies. The lower limit of detection for the GFPC was approximately 10 percent of the 800,000 dpm spikes. Given

the low levels of $^{14}CO_2$ produced in the mineralization studies any ^{14}C-transformation products formed in the test flasks may have been present at concentrations lower than the detection limit.

The low recovery of ^{14}C activity as $^{14}CO_2$ or ^{14}C-organics in the isodrin composting study indicates that isodrin was not mineralized or volatilized. Data from this experiment suggest that isodrin is environmentally persistent even under thermophilic composting conditions.

5.3 CONCLUSIONS

Mineralization data obtained in the present study indicate that biotransformation and mineralization played minor roles in the fate of the radiolabeled test compounds in test soils, despite the presence of metabolically active populations of heterotrophic microorganisms in uncontaminated soil and soil nos. 3224, 3256, and 3215. These data confirm earlier findings that DIMP, dieldrin, isodrin, DBCP, and PCPMSO are environmentally persistent in soil. In the case of soil nos. 3219 and 3204, high concentrations of soil contaminants apparently severely inhibit microbial activity.

Given the favorable microbial growth conditions (optimal soil moisture, adequate oxygen tension, and 22°C temperature) and the low levels of mineralization observed in test flasks, loss of DIMP, dieldrin, isodrin, DBCP, and PCPMSO as a result of microbial metabolism from in situ RMA soils is expected to be minimal.

SECTION 6

LITERATURE CITED

Bateman, L. and K.R. Hargrove, 1954a. Oxidation of Organic Sulfides I. Interaction of Cyclohexyl Methyl Sulfide with Hydroperoxides in Alcohols. Proc. Roy. Soc., A224:389-396. As cited in Miller et al., 1976.

Bateman, L. and K.R. Hargrove, 1954b. Oxidation of Organic Sulfides. II. Introduction of Cyclohexyl Methyl Sulfide with Hydroperoxides in Hydrocarbons. Proc. Roy. Soc., A224:399-411. As cited in Miller et al., 1976.

Berkowitz, A.E., M.M. Goyer, J.C. Harris, W.J. Lyman, R.A. Horne, L.H. Nehen, and J.E. Harrison, 1978. Chemistry, Toxicology and Potential Environmental Effects of Selected Organic Pollutants. Final Report, U.S. Army Contract DAMD17-77-C-7073.

Bixby, M.W., G.M. Boush, and F. Matsumura, 1971. Degradation of Dieldrin to Carbon Dioxide by a Soil Fungus Trichoderma knongii. Bull. Environ. Contam. Toxicol., 6:49-494.

Brooks, G.T., 1974. Chlorinated Insecticides. CRC Press, Cleveland, Ohio.

Carter, G.E., J.T. Ligon, and M.B. Riley, 1984. DBCP Residue Studies in Soil and Well Water from Two South Carolina Peach Orchards. Water, Air, and Soil Pollution, 22:201-208.

Castro, C.E. and N.O. Belser, 1968. Biodehalogenation and Reductive Dehalogenation of the Biocides Ethylene Bromide, 1,2-Dibromo-3-Chloropropane, and 2,3-Dibromobutane in Soil. Environ. Sci. Technol., 2:779-783.

Castro, T.F. and T. Yoshida, 1971. Degradation of Organotin Insecticides in Flooded Soils in the Philippines. J. Agric. Food Chem., 19:1168-1170.

Cogley, D.R. and W. Goy, 1978. Readily Available Data on 169 Compounds Associated with Operations at Rocky Mountain and Pine Bluff Arsenals. United States Army Contract DAMD 17-77-C-7050. Rocky Mountain Arsenal Information Center, Commerce City, Colorado.

Crowley, G.T. and E.P. Lichtenstein, 1970. Growth Inhibition of Soil Fungi by Insecticides and Annulment of Inhibition by Yeast Extract or Nitrogenous Nutrients. J. Gen. Microbiol., 62:27-34.

Daughton C.G., A.M. Cood, and M. Alexander, 1979a. Bacterial Conversion of Alkyphosphonates to Natural Products via Carbon-Phosphorus Bond Cleavage. J. Agric. Food Chem., 27:1375-1381.

Daughton, C.G., A.M. Cood, and M. Alexander, 1979b. Biodegradation of Phosphonate Toxicants Yields Methane or Ethane on Cleavage of C-P Bond. REMS Microbiology Letters, 5:91-92.

Eichelberger, J.W. and J.J. Litchenburg, 1971. Persistence of Pesticides in River Waste. Environ. Sci. Technol., 5:501-504.

Elsner, E.O., D. Beinek, W. Klein, and F. Korte, 1977. Beitragezur Okologiachen Chemi Lii. Yerteilung und Umdwandlung von Aldrin-^{14}C, Heptachlor-^{14}C, and Lindan-^{14}C in der Grunalge Chlorella purenoidosa. Chemsphere, 1:247-250. As cited in The Degradation of Selected Pesticides in Soil: A Review of the Published Literature. 1977. U.S. Dept. of Commerce, PB-272353.

Guensi, W.D., W.E. Beard, R.A. Bowman, and S.R. Olsen, 1979. Plant Toxicity and Soil Transformations of Soil Incorporated Sulfur Compounds. USDA-SEA Agricultural Research, DTIC Report No. AD-A091311, Fort Collins, Colorado.

Halvorson, H.M., Ishague, R.A. Bowman, and S.R. Olsen, 1979. Biodegradability Test for Insecticides. Can. J. Microbiol., 17:585-591.

Kresze, G., E. Ropte, and B. Schraeder, 1965. Structure of Organo Sulfur Compounds X. Infrared and Raman Spectra of Methyl Aryl Sulfides, Sulfoxides, and Sulfones. Spectrochim Acta, 2:1633-1654. As cited in Miller et al, 1976.

Lehmicke, L.G., R.T. Williams, and R.L. Crawford, 1979. ^{14}C-Most-Probable-Number Method for Enumerations of Active Heterotrophic Microorganisms in Natural Waters. Appl. Environ. Microbiol., 38:644-649.

Matsumura, F. and G.M. Boush, 1967. Dieldrin: Degradation by Soil Microbes. Science, 156:959-961.

Matsumura, F., V.G. Khanvilkar, K.C. Patil, and G.M. Boush, 1971. Metabolism of Endrin by Certain Soil Microorganisms. J. Agric. Food Chem., 19:27-31.

Matsumura, F., K.C. Patil, and G.M. Bouch, 1970. Formation of Photodieldrin by Microorganisms. Science, 170:1206-1207.

Miller, T.A., D.H. Rosenblatt, J.C. Dacre, J.G. Pearson, R.K. Kulkami, J.L. Welch, D.R. Cogley, and G. Woodward, 1976. Problem Definition Studies on Potential Environmental Pollutants IV: Physical, Chemical, Toxicological, and Biological Properties of Benzene, Toluene, Xylenes, and p-Chlorophenyl Methyl Sulfide, Sulfoxide, and Sulfone. U.S. Army Medical Bioengineering Research and Development Laboratory, Technical Report 7605. Frederick, Maryland.

Mueller, W.P. and F. Korte, 1972. Contributions to Ecological Chemistry CII Microbial Degradation of Benzo-a-pyrene, Monolinuron, and Dieldrin in Waste Composting. Chemosphere, 4:195-198.

Murado-Garcia, M.A. and G. Balvia-Marcos, 1972. Metabolism of Aldrin by Penicilium funiculosum. Rev. Agroquim. Technol. Ailment, 13:559-556.

Nash, R.G. and W.G. Harris, 1973. Clorinated Hydrocarbon Insecticide Residues in Crops and Soils. J. Environ. Qual., 2:269-273.

Nash, R.G. and E.A. Woolson, 1967. Persistence of Clorinated Hydrocarbon Pesticides in Soils. Science, 157:924-927.

Nash, R.G. and E.A. Woolson, 1968. Distribution of Clorinated Insecticides in Cultivated Soil. Soil Sci. Soc. Amer. Prac., 32:525-527.

Nelson, S.J., M. Iskander, M. Volz, S. Khalifa, and R. Haberman, 1981. Studies of DBCP in Subsoils. Science of the Total Environment, 21:35-40.

O'Donovan, P.A. and J.E. Woodward, 1977. Investigations of the Environmental Fate and Phytotoxicity of DIMP and DCPD. Defense Technical Information Center, Aerojet Ordinance and Manufacturing Report 1953-01(01)FP. Alexandria, Virginia. pp. 22,304-26,145.

Overberger, C.G. and R.W. Cummins, 1953a. Mechanism of the Oxidation of P,P'-Dichlorobenzyl Sulfide by Perbenzoic and Para Substituted Peroxybenzoic Acids. J. Amer. Chem. Soc., 75:4250-4254. As cited in Miller et al. , 1976.

Overberger, C.G. and R.W. Cummins, 1953b. Kinetics and Mechanism of the Oxidation of P,P'-Dichlorobenzyl Sulfide by Hydrogen Peroxide. J. Amer. Chem. Soc., 75:4783-4787. As cited in Miller et al., 1976.

Patil, K.C. and F. Matsumura, 1979. Degradation of Endrin, Aldrin, and DDT by Soil Microorganisms. Appl. Microbiol., 19:879-881.

Reddy, G. and M.A.Q. Khan, 1975. Fate of Photodieldrin Under Various Environmental Conditions. Bull. Environ. Contam. Toxicol., 13:64-72.

Rosenblatt, D.H., T.A. Miller, J.C. Dacra, I. Muul, and D.R. Cogley, 1975. Problem Definition Studies in Potential Environmental Pollutants II. Physical, Chemical, Toxicological, and Bioengineering Research and Development Laboratory, Rocky Mountain Arsenal Information Center. Commerce City, Colorado.

Sanborn, J.R. et al., 1977. The Degradation of Selected Pesticides in Soil: A Review of the Published Literature. U.S. Environmental Protection Agency, 600/9-77-022.

Sanderson, J.L. and E.W. Swift, 1972. Oxidation of Aryl Alkyl Sulfoxides to Aryl Alkyl Sulfones. United States Patent No. 3,699,171. 17 October 1972: C.A. 78:15794W. As cited in Miller et al., 1976.

Sharom, M.S., 1980. Water Res., 14:1089-1093.

Spangord, R.J., C. Tsong-Wen, and W.R. Mabey, 1979. Studies on the Environmental Fate of DIMP and DCPD. Final Report, U.S. Army Contract 17-87-C-8053, DTICAD-A078236.

Tabak, H.H. et al., 1981. J. Water Pollution Control Fed., 53:1503-1518.

Tu, C.M., J.R.W. Miles, and C.R. Harris, 1968. Soil Microbial Degradation of Aldrin. Life Sci., 7:311-322.

United States Environmental Protection Agency. Test Methods for Evaluating Solid Wastes. U.S. EPA SW846.

Yasu, K. and N.K. Roy, 1986. Hydrolysis, Phytotoxicity, and Photolysis Studies on Q,O-bis(2,4,5-trichlorophenyl) Methyl Phosphonate, a Potential Fungicide. J. Agric. Biol. Chem., 49:307- 310.

Yerweij, A., C.E.A.M. Degenhardt, and H.L. Boter, 1976. The Occurrence and Determination of PCH_3-Containing Compounds in Surface Water. Chemosphere, 3:115-124.

Vockel, O. and F. Korte, 1974. Beitragesur Okologischen Chemie LXXX. Yersuckezum Mikrobiellen Abbau von Dieldrin and 2,2-Dichlorobiphenyl. Chemosphere, 3:177-182.

Williams, R.T., P.S. Ziegenfuss, and P.J. Marks, 1988. Field Demonstration -- Composting of Explosives-Contaminated Sediments at the Louisiana Army Ammunition Plant (LAAP). United States Army Toxic and Hazardous Materials Agency Report No. AMXTH-IR-TE-88242. Aberdeen Proving Ground, Maryland.

Zeleznick, L.D., F.C. Nyers, and E.B. Titchener, 1963. Growth of E. Coli on Methyl and Ethyl Phosphonic Acids. Biochem. Biophys. Scin., 78.

APPENDIX A

ANALYTICAL METHODS

SEMIVOLATILE ORGANICS IN SOIL/SEDIMENT SAMPLES BY GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS)

1. APPLICATION

This method is applicable to the semiquantitative determination of the following compounds in environmental soil/sediment samples by GC/MS:

- Aldrin
- Atrazine
- Chlordane
- p-Chlorophenylmethyl sulfide (PCPMS)
- p-Chlorophenylmethyl sulfone ($PCPMSO_2$)
- p-Chlorophenylmethyl sulfoxide (PCPMSO)
- Dibromochloropropane (DBCP)
- Dicyclopentadiene (DCPD)
- 4,4'-DDE
- 4,4'-DDT
- Dieldrin
- Diisopropylmethylphosphonate (DIMP)
- 1,4-Dithiane (Dithiane)
- Dimethylmethylphosphonate (DMMP)
- Endrin
- Hexachlorocyclopentadiene (HCCPD)
- Isodrin
- Malathion
- 1,4-Oxathiane (Oxathiane)
- Parathion
- Supona
- Vapona

A. TESTED CONCENTRATION RANGE

The tested concentration ranges of the compounds examined in "standard soil" are as follows:

Analyte	Tested Concentration Range (µg/g)*
Aldrin	0.25 to 99.7
Atrazine	0.25 to 99.7
Chlordane	0.25 to 99.7
PCPMS	0.25 to 99.7
PCPMSO	0.25 to 99.7
$PCPMSO_2$	0.25 to 99.7
DBCP	0.25 to 99.7
DCPD	0.25 to 99.7
4,4'-DDE	0.25 to 99.7
4,4'-DDT	0.25 to 99.7
Dieldrin	0.25 to 99.7
DMMP	0.25 to 99.7
DIMP	0.25 to 99.7
Dithiane	0.25 to 99.7
Endrin	0.25 to 99.7
HCCPD	0.25 to 99.7
Isodrin	0.25 to 99.7
Malathion	0.25 to 99.7
Oxathiane	0.25 to 99.7
Parathion	0.25 to 99.7
Supona	0.25 to 99.7
Vapona	0.25 to 99.7
2-Chlorophenol-D4	0.25 to 99.7
1,3-Dichlorobenzene-D4	0.25 to 99.7
Diethylphthalate-D4	0.25 to 99.7
Di-n-Octylphthalate-D4	0.25 to 99.7

B. SENSITIVITY

The extracted ion current area count responses at the "standard soil" detection limits (Sec. 1.C) are:

Analyte	RRT*	Area Counts	Quantitation Ion
Aldrin	1.107	3,650	263
Atrazine	0.983	9,220	200
Chlordane	1.180	1,499	373
PCPMS	0.660	18,670	158
$PCPMSO_2$	0.828	12,600	159
PCPMSO	0.857	4,390	175
DBCP	0.500	12,700	157
DCPD	0.445	5,340	132
4,4'-DDE	1.209	2,170	246
4,4'-DDT	1.287	2,000	235
Dieldrin	1.212	4,900	79
DIMP	0.488	40,000	123
Dithiane	0.481	26,900	120
DMMP	0.283	300,000	94
Endrin	1.230	2,970	263
HCCPD	0.711	12,200	237
Isodrin	1.138	1,800	193
Malathion	1.103	3,900	173
Oxathiane	0.284	17,900	104
Parathion	1.115	7,300	137
Supona	1.156	5,670	267
Vapona	0.650	44,250	109
2-Chloro-phenol-D4	0.405	16,300	132
1,3-Dichloro-benzene-D4	0.420	29,400	150
Diethyl-phthalate-D4	0.886	18,200	153
Di-n-octyl-phthalate-D4	1.439	13,700	153

*RRT = Relative retention time (relative to phenanthrene-D10 which had a retention time of 19.54 minutes).

C. DETECTION LIMITS AND CERTIFIED RANGES

The detection limits in "standard soil", calculated according to the U.S. Army Toxic and Hazardous Materials Agency (USATHAMA) detection limit program with 90-percent confidence limits (USATHAMA, 1982), are:

Analyte	Detection Limit (ug/g)	Upper Certified Range (ug/g)
Aldrin	0.9	99.7
Atrazine	0.7	99.7
Chlordane	1	100
PCPMS	0.3	49.9
$PCPMSO_2$	0.4	49.9
PCPMSO	0.3	99.7
DBCP	0.3	49.9
DCPD	0.3	25.0
4,4'-DDE	0.3	99.7
4,4'-DDT	0.4	99.7
Dieldrin	0.3	99.7
DIMP	0.5	9.97
Dithiane	0.3	99.7
DMMP	2	9.97
Endrin	0.7	49.9
HCCPD	1	49.9
Isodrin	0.3	99.7
Malathion	0.6	2.5
Oxathiane	0.3	99.7
Parathion	0.7	99.7
Supona	0.5	9.97
Vapona	0.3	25.0
2-Chlorophenol-D4	0.3	25.0
1,3-Dichlorobenzene-D4	0.3	99.7
Diethylphthalate-D4	0.3	49.9
Di-n-octylphthalate-D4	0.6	99.7

D. INTERFERENCES

Solvents, reagents, glassware, and other sample processing hardware may yield discrete artifacts and/or elevated baselines causing misinterpretation of chromatograms. All of these materials must be demonstrated to be free from interferences under the conditions of the analysis by running method blanks. Specific selection of reagents and purification of solvents by distillation in all-glass systems may be required. No interferences were encountered in the "standard soil" analysis.

E. ANALYSIS RATE

After extraction and instrument calibration, one analyst can analyze five samples in an 8-hour day.

2. CHEMISTRY

A. CHEMICAL ABSTRACT SERVICE (CAS) REGISTRY NUMBERS

Analyte	CAS Registry Number
Aldrin	309-00-2
Atrazine	1912-24-9
Chlordane	57-74-9
PCPMS	123-09-1
$PCPMSO_2$	934-73-6
PCPMSO	98-57-7
DBCP	96-12-8
DCPD	77-73-6
4,4'-DDE	75-55-9
4,4'-DDT	50-29-3
Dieldrin	60-57-1
DIMP	1445-75-6
Dithiane	505-29-3
DMMP	756-79-6
Endrin	72-20-8
HCCPD	77-47-4

Analyte	CAS Registry Number
Isodrin	465-73-6
Malathion	121-75-5
Oxathiane	15980-15-1
Parathion	56-38-2
Supona	2701-86-2
Vapona	62-73-7

B. CHEMICAL REACTIONS

A measured weight of soil/sediment is extracted with methylene chloride using a Soxhlet extractor. The methylene chloride extract is dried and concentrated. Chromatographic conditions are described which permit the separation and measurement of the parameters in the extract. Qualitative identification is performed using the retention time and the relative abundance of characteristic ions. Semiquantitative analysis is performed using internal standard techniques with a single characteristic ion.

3. APPARATUS

A. INSTRUMENTATION

1. Hewlett-Packard 5985A [gas chromatograph/mass spectrometer/data system (GC/MS/DS)] with dual electron impact/chemical ionization (EI/CI) source (or equivalent). The system is operated in the EI mode and tuned according to EPA criteria for DFTPP (see Table 1).
2. Hewlett-Packard Model 5710 (or equivalent) gas chromatograph equipped with a flame-ionization detector is used for the GC-Screens. The GC is coupled to a strip-chart recorder.

Table 1. DFTPP Mass-Intensity Specifications

Mass	Intensity Required
51	8-82 percent of mass 198
68	<2 percent of mass 69
69	11-91 percent of mass 198
70	<2 percent of mass 69
127	32-59 percent of mass 198
198	base peak, 100 percent abundance
199	4-9 percent of mass 198
275	11-30 percent of mass 198
441	44-110 percent of mass 443
442	30-86 percent of mass 198
443	14-24 percent of mass 442

Source: EPA Method 1625 Revision B, January 1985.

B. GAS CHROMATOGRAPH/MASS SPECTROMETER (GC/MS) PARAMETERS, HARDWARE, GLASSWARE, AND REAGENTS

1. GC/MS

   a. Column: 30 m by 0.25 mm (id) DB5 (25-µm coating) fused silica held isothermal at 45°C for 4 min and then programmed at 10°C/min to 280°C.

   b. Conditions: Injector port--280°C; transfer line--290°C; carrier gas (helium) at about 30 cm/sec; electron energy--70 ev.

2. GC Screen

   a. Column: 10 m by 0.53 mm (id) 5-percent phenylmethyl silicone-fused silica column held isothermal at 90°C for 4 min and temperature programmed at 8°C/min to 280°C. Any column run under appropriate conditions which provides the GC Screen data outlined in Sec. 5.H may be substituted.

   b. Conditions: Injector port--250°C; detector--300°C; carrier gas (nitrogen) at about 4 mL/min.

3. Injection Volume: 2.0 uL

4. Retention Times (GC/MS): See Sec. 3.B above.

C. HARDWARE/GLASSWARE

1. Sample containers, as appropriate.
2. Soxhlet extractors and associated equipment.
3. K-D concentrators, Snyder columns and heated water bath.
4. Volumetric flasks and pipettes as necessary.

D. CHEMICALS

1. Dichloromethane, acetone and toluene: Burdick and Jackson pesticide quality or equivalent.
2. Analytical reference standards of each analyte. Malathion, Parathion, Azodrin, Supona, Vapona, and Atrazine are from EPA (Reference Standards Repository, RTP-N.C.); all others are USATHAMA SARMS or interm SARMS.

3. Decafluorotriphenylphosphine (DFTPP).
4. 1,3-Dichlorobenzene-D4, diethylphthalate-D4, di-n-octylphthalate-D4, and 2-chlorophenol-D4 as surrogates from USATHAMA as SARMS.
5. Phenanthrene-D10 as an internal standard (Cambridge Isotope Labs).
6. Phenanthrene as GC Screen standard (Aldrich Chemical).
7. Anhydrous sodium sulfate (dichloromethane rinsed).

4. STANDARDS

A. INSTRUMENT CALIBRATION STANDARDS

1. Individual stock calibration standards of the target compounds and surrogates were prepared by weighing approximately 100 milligrams (mg) of each compound separately into a 10-mL volumetric flask and diluting to volume with methylene chloride. These stock calibration standards had concentrations of approximately 10,000 micrograms per milliliter (µg/mL) of each compound.
2. A composite stock calibration standard A, containing each compound at a concentration of 150 µg/mL, was prepared by taking 1.5 mL of each stock calibration standard and diluting to 100 mL with methylene chloride.
3. The stock calibration internal standard was made by weighing 100 mg of phenanthrene-D10 and diluting to volume with methylene chloride into a 10-mL volumetric flask. The stock calibration internal standard has a concentration of 10,000 µg/mL.
4. Take 2 mL of the stock calibration internal standard and dilute it to volume in a 10-mL volumetric flask to yield a 2,000 µg/mL working internal standard solution.
5. Prepare the dilute composite calibration standards B, C, D, E, and F using the dilution scheme outlined in Table 2. Each dilution is made by taking appropriate aliquots of a

Table 2. Preparation of Composite Calibration Standards

Composite Calibration Standard Used	Volume Used (mL)	Final Volume (mL)	Composite Calibration Standard Prepared	Prepared Standard Analyte Concentration (µg/mL)
-	--	--	A	150
A	50	100	B	75
B	50	100	C	37.5
B	20	100	D	15
C	20	100	E	7.5
E	50	100	F	3.75

Source: ESE, 1985.

calibration standard and diluting to final volume with methylene chloride.

B. INITIAL INSTRUMENT CALIBRATON

1. The GC/MS is tuned to meet EPA criteria for DFTPP as given in Table 1.
2. To calibrate the instrument and establish the linear range for the target compounds and surrogates, separate 1.0-mL aliquots of the appropriate composite calibration standards A, C, E, and F are spiked with 25 microliters (µL) of the working internal standard solution, mixed well, and 2 µL injected and analyzed. A blank containing 1 mL of methylene chloride solvent spiked with 25 µL of the working internal standard solution is also analyzed. Standards are analyzed in order of increasing concentration. A minimum of three calibration standards and one blank are analyzed to bracket the certified range for each analyte. The initial calibration data are utilized to establish response factors and calibration curves.

C. DAILY CALIBRATION STANDARD

1. The GC/MS is tuned to meet EPA criteria for DFTPP as given in Table 1.
2. A one-point calibration standard is analyzed daily to calibrate the instrument and establish response factors.
3. A 1-mL aliquot of the composite calibration standard is spiked with 25 µL of the working internal standard solution, well mixed, and 2 µL injected and analyzed. This standard is utilized to establish daily response factors for each target and surrogate compound. If the daily response factors obtained from the analysis of composite calibration standard C differ (using "%D" as defined below) from the average RFs by more than 25 percent for any of the

surrogates, then a new standard curve and new set of average response factors must be made. If the daily calibration is within the acceptable "%D" window, then analysis of the sample extracts can proceed. The percent difference (%D) calculation is [(daily RF-average RF)/average RF] x 100.

D. CERTIFICATION CONTROL SPIKES

1. An uncontaminated natural soil from the Rocky Mountain Arsenal area was used as the "standard soil." The soil was mixed, and 15-gram (g) aliquots were placed into clean 125-mL beakers.
2. Thirty grams of sodium sulfate were added to each beaker. The contents was mixed and then quantitatively transferred to separate sample reservoirs of Soxhlets. The certification control samples were then spiked as described in Table 3 and allowed to equilibrate for 1 hour. After equilibration the certification control spikes are analyzed beginning with Sec. 5.F.

(Note: The certification control spike extracts final volumes were 1.0 mL for control spike samples 1 through 7, 10 mL for control spike samples 8 and 9, and 50 mL for control spike sample 10. The final volumes noted will keep the analytes and surrogates within the 3.75 ug/mL to 150 ug/mL GC/MS working standard concentration range. The use of dilutions is legitimate in view of the GC-FID screen which will be used for real samples. For the purpose of certification, no GC-FID screens need be employed.)

Table 3. Certification Control Spike Solution

Control Spike Sample Number	Composite Calibration Standard Used	Volume of Composite Calibration Standard Used (mL)	Migrograms of Each Target Compound Added to the Control Spike Sample	Concentration of Each Target Compound in the Control Spike Sample (µg/g)*
1 (Blank)	None	None	0	0
2	F	1.0	3.75	0.25
3	E	1.0	7.5	0.50
4	D	1.0	15	1.0
5	C	1.0	37.5	2.5
6	B	1.0	75	5.0
7	A	1.0	150	10
8	A	2.5	375	25
9	A	5.0	750	50
10	A	10.0	1,500	100

*Based upon 15 g of "standard soil" being spiked.

Source: ESE, 1985.

5. PROCEDURE

A. Prepare daily composite stock surrogate and daily composite working surrogate solutions (to be used in the analysis of environmental samples) as follows. Place 1.5 mL of each of the four surrogate stock calibration standards into a 100-mL volumetric flask and dilute to volume with methylene chloride. This daily composite working surrogate solution contains each of the four surrogate compounds at a concentration of 150 µg/mL.

B. Place 15 g of standard soil into a clean 125-mL beaker. This will be the daily method blank.

C. Place 15 g of the environmental samples into separate 125-mL beakers.

D. Add 30 g of sodium sulfate to each beaker, mix well, then quantitatively transfer the contents of each beaker to separate sample reservoirs of Soxhlets.

E. Spike each sample in the Soxhlets with 1.0 mL of the daily composite working surrogate solution. This produces a spiking concentration of 10 µg/g for each surrogate in each sample. Allow the spike to equilibrate for 1 hour.

F. Add 300 mL of methylene chloride and a few clean boiling chips to the solvent reservoir of each Soxhlet and allow the samples to extract for a minimum of 8 hours.

G. The extracts are concentrated to a temporary final volume of 10 mL using a K-D setup.

H. Screen the extracts by GC/FID using GC-FID screening. The GC-FID attentuation is adjusted so that a 2-µL injection of the 15-µg/mL phenanthrene standard gives about 50-percent full-scale deflection. The sample extracts from Sec. 5.G above are analyzed by GC-FID using 2-mL injections to determine whether the extracts will require either concentration or dilution such that the largest GC-FID peaks will not exceed the highest (150 µg/mL) GC/MS analyte standards. For example, if there are no GC-FID peaks greater than the 15-µg/mL screen standard, the sample extract can be concentrated to 1.0-mL final volume; this will allow the detection limits to be met while not generating analyte peaks greater than the highest GC/MS standards.

I. Perform daily GC/MS calibration as described in Sec. 4.C.

J. After determining the final extract volume from the GC-FID screening (Sec. 5.H), remove a 1.0-mL extract aliquot, spike it with 25 µL of the working internal standard solution, mix well, then inject a 2-µL aliquot.

K. Calculate the concentration of any detected target of surrogate compound using response factors as described in Sec. 6.

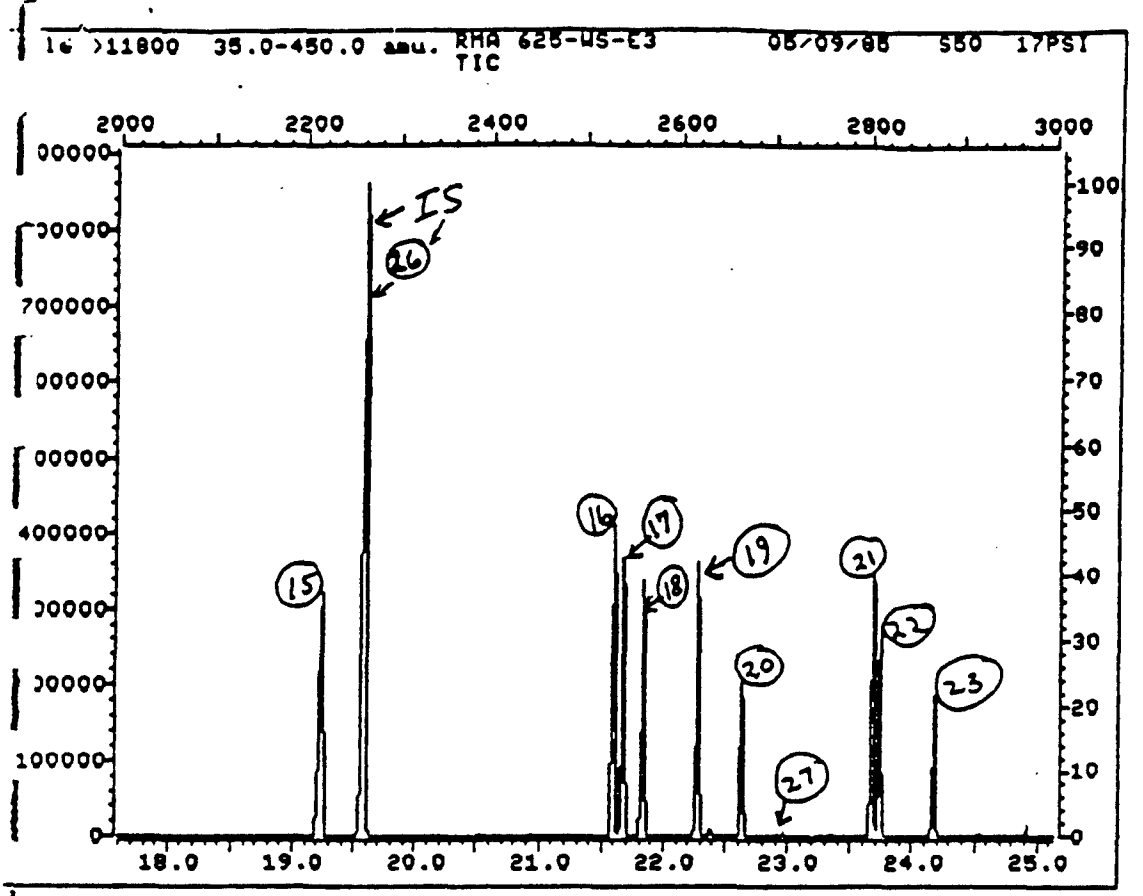
L. Unknown GC/MS peaks will be tentatively identified by computer-assisted comparison to the EPA/NBS/NIH mass spectral library (or equivalent). The mass spectroscopist will use the probability-based matching criteria to assign probabilities of correct structural assignment. Hardcopy mass spectra of all unknowns will be provided with the report.

The largest five unknown peaks which are present in excess of 10 percent of the area of the m/e 188 peak for phenanthrene-D10 internal standard will be library searched. Unknowns will be

ATTACHMENT 2

Reconstructed Total Ion Current Profile of
Composite Calibration Standard C (37.5 micrograms per milliliter)

Analyte	Analyte Number on Chromatogram
MP	1
athiane	2
-Chlorophenol-D4	3
,3-Dichlorobenzene-D4	4
CPD	5
ithiane	6
LMP	7
BCP	8
apona	9
PMS	10
CCPD	11
$CPMSO_2$	12
CPMSO	13
iethylphthalate-D4	14
trazine	15
alathion	16
ldrin	17
arathion	18
sodrin	19
upona	20
,4'-DDE	21
ieldrin	22
ndrin	23
,4'-DDT	24
i-n-octylphthalate-D4	25
henanthrene-D10	26
hlordane	27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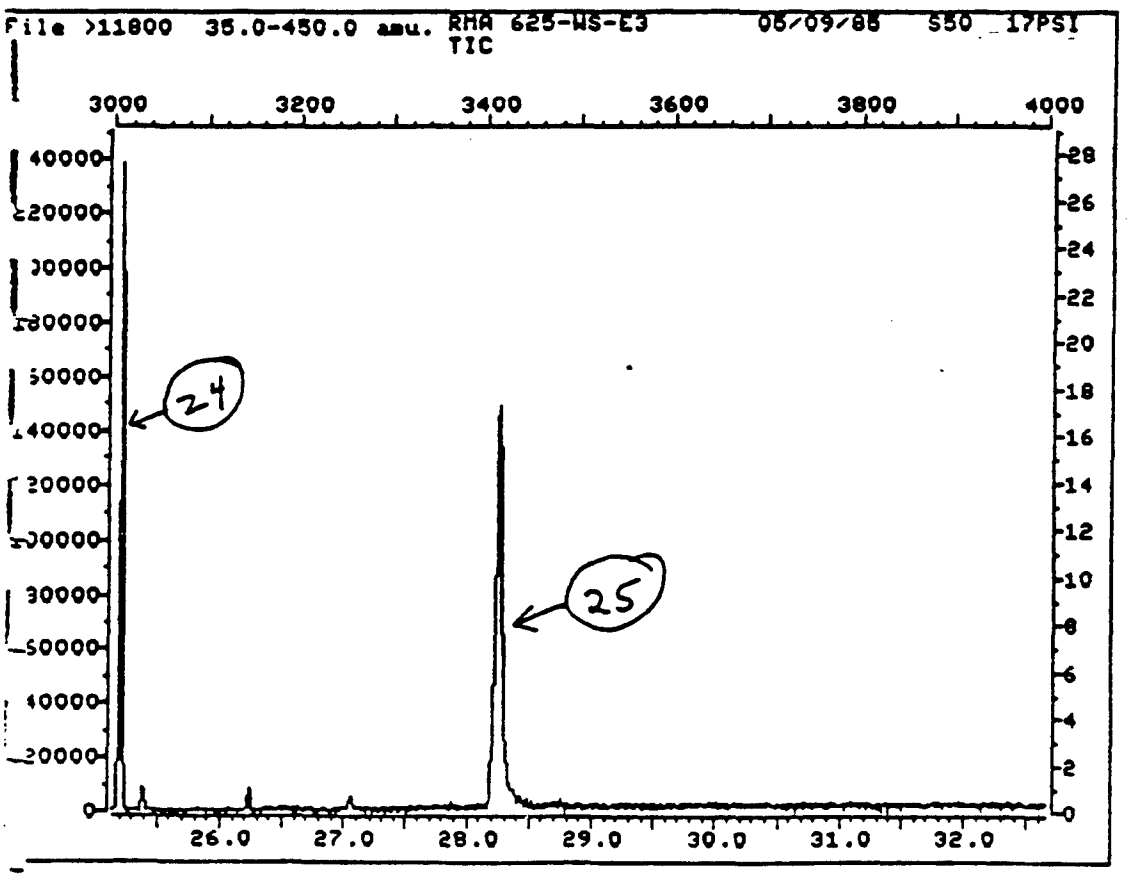
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APPENDIX B

ESE/EBASCO ANALYTICAL DATA

Table 36-1-4. Concentrations of Target Analytes Above Detection Limits in Site 36-1 Soil Samples (Page 1 of 10)

Bore Number	3199	3199	3199	3200	3200	3201	3201	3202	3202	3203	3203	3203	3204
Depth (ft)	0-1	4-5	9-10	0-1	4-5	0-1	4-5	0-1	4-5	0-1	4-5	7-8	0-1
Geologic Material	Silty Sand	Very Fine Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Sandy Silt	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand
AIR MONITORING													
PID*	BKD	BKD	BKD	2	100	BKD	BKD	BKD	BKD	BKD	BKD	BKD	BKD
SOIL CHEMISTRY													
Volatiles (µg/g)													
$CHCL_3$	NA	BDL	BDL	NA	BDL	NA	BDL	NA	BDL	NA	BDL	0.8	NA
Semivolatiles (µg/g)													
Aldrin	BDL	BDL	BDL	BDL	BDL	40	BDL	BDL	BDL	300	BDL	BDL	300
Dieldrin	BDL	BDL	BDL	BDL	BDL	8	BDL	4	BDL	BDL	BDL	BDL	400
Endrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	200
Chlordane	BDL	BDL	BDL	BDL	BDL	6	BDL	10	BDL	BDL	BDL	BDL	100
Isodrin	BDL	BDL	BDL	BDL	BDL	2	BDL	BDL	BDL	200	BDL	BDL	40
CPMS	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	200
CPMSO	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6
$CPMSO_2$	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	6
DBCP (µg/g)	BDL	BDL	BDL	BDL	BDL	0.007	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Metals (µg/g)													
Cadmium	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.4	BDL	BDL	BDL	BDL	2.2
Chromium	21	7.0	17	8.0	BDL	23	12	31	16	17	16	14	17
Copper	11	6.0	10	6.0	6.0	6.0	8.0	44	16	20	16	13	22
Lead	BDL	BDL	BDL	BDL	BDL	BDL	BDL	33	BDL	43	BDL	BDL	26
Zinc	40	32	43	29	31	32	47	97	41	BDL	38	BDL	56
Arsenic (µg/g)	10	5.5	6.5	BDL	BDL	36	13	44	BDL	20	BDL	BDL	220
Mercury (µg/g)	BDL	BDL	BDL	BDL	BDL	0.23	BDL	0.53	0.080	0.51	BDL	BDL	1.1

Table 36-1-4. Concentrations of Target Analytes Above Detection Limits in Site 36-1 Soil Samples (Continued, Page 3 of 10)

Bore Number	3211	3211	3212	3213	3213	3214	3214	3215	3215	3216	3217	3218	3219
Depth (ft)	0-1	3-4	0-1	0-1	3-4	0-1	4-5	0-1	4-5	0-1	0-1	0-1	0-1
Geologic Material	Silty Sand	Saturated Silty Sand	Silty Sand	Very Fine Sand/Sandy Silt	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Silty Sand	Sandy Sand	Silty Sand	Silty Sand	Very Fine Sand/ Nonsoil
AIR MONITORING													
PID*	BKD	BKD	BKD	5	30	25	27	45	BKD	BKD	BKD	BKD	BKD
SOIL CHEMISTRY													
Volatiles (µg/g)	NA	BDL	NA	NA	BDL	NA	BDL	NA	BDL	NA	NA	NA	NA
Semivolatiles (µg/g)													
Aldrin	BDL	BDL	BDL	0.8	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	50
Dieldrin	BDL	BDL	BDL	2	BDL	BDL	BDL	BDL	BDL	BDL	1	BDL	30
Chlordane	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	10
DIMP	BDL	BDL	BDL	BDL	BDL	BDL	BDL	4	BDL	BDL	BDL	BDL	BDL
Isodrin	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	3
CPMS	BDL	BDL	BDL	BDL	0.5	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
DBCP (µg/g)	BDL	BDL	BDL	BDL	BDL	BDL	0.096	BDL	0.11	BDL	BDL	BDL	BDL
Metals (µg/g)													
Cadmium	BDL	BDL	0.60	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	1.3	1.5
Chromium	16	14	14	17	14	14	12	22	BDL	15	14	16	BDL
Copper	12	11	15	20	15	15	12	19	8.0	11	15	22	63
Lead	BDL	BDL	BDL	23	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL	BDL
Zinc	59	55	46	49	45	40	BDL	60	BDL	56	41	47	BDL
Arsenic (µg/g)	19	11	53	BDL	BDL	12	BDL	8.4	BDL	37	29	140	87
Mercury (µg/g)	0.050	BDL	0.12	BDL	BDL	0.13	BDL	BDL	BDL	0.17	0.26	0.49	1.5